JOURNAL OF AGRICULTURAL RESEARCH

VOL. 47 WASHINGTON, D.C., OCTOBER 15, 1933 NO. 8

THE PREVENTION OF ANEMIA IN SUCKLING PIGS, WITH OBSERVATIONS ON THE BLOOD PICTURE[1]

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INTRODUCTION

That nutritional anemia may occur in suckling pigs under farm as well as under experimental conditions is now well known. The pig, however, is not the only class of farm animals subject to this disease. The possibility of the occurrence of anemia in calves has been recognized at the Kansas station (*3*),[2] and Becker, Neal, and Shealy (*1*) have shown that this disease is quite common in cattle in certain sections of Florida. Hamilton and Card (*22*) and Elvehjem and Hart (*12*) have produced severe anemia in chicks by feeding them milk on screen floors. Hart, Elvehjem, Kemmerer, and Halpin (*16*) later showed that the feeding of milk and yellow corn to chicks, from hatching, readily produced anemia. However, anemia does not seem to be of any practical consideration in poultry production, and according to the work of Thomas and Wheeler (*34*) the same seems to be true of sheep.

In the case of the pig, however, it is now recognized that, under present-day methods of swine management, the disease is responsible for the loss of no inconsiderable number of animals before weaning time. Any environmental, farm, or experimental conditions that restrict the little pigs to a diet of milk alone for a period of 3 to 5 weeks after farrowing will usually produce the disease. While the etiology of the disease is still somewhat in doubt, investigations during the last 10 years have established the fact that the inclusion in the milk diet of the pig of very small amounts of soluble iron and copper salts, or of iron salts alone, will entirely prevent the onset of the disease.

WORK OF OTHER INVESTIGATORS

Although Doyle, Mathews, and Whiting (*10*) quote Braasch as reporting an extraordinarily high death rate of pigs due to anemia, in Schleswig-Holstein in 1890, and McGowan and Crichton (*25*) call attention to the probability that some of the swine diseases reported in the literature in the past may have been, in reality, anemia, practically all the investigations concerned with this disease in swine have been carried out during the last 10 years.

McGowan and Crichton (*25*) recognized the disease in England prior to 1923. The external symptoms as well as the gross lesions

[1] Received for publication May 26, 1933; issued October 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 561.

and microscopic findings at necropsy described by these investigators have been confirmed by Doyle, Mathews, and Whiting (*10*), as well as by others.

In 1924 McGowan and his associates (*24, 26*) produced anemia in suckling pigs experimentally by confining them indoors, without exercise, and with access only to the iron-poor diet of the sow. McGowan concluded that the disease resulted from an iron deficiency in the diet. He said that the disease could be readily prevented or cured by the inclusion of ferric oxide in the sow's feed. It was pointed out that the favorable effect upon the pigs of this procedure was probably due, not to an increase in the iron content of the sow's milk, but to the small amount of iron actually consumed by the pigs from the iron-rich feed and feces of the sow. The practicability of such a procedure for preventing anemia may be questioned, however, since Hart and his associates (*6, 17, 18*) failed to obtain beneficial results from the administration of iron oxide directly to the little pigs, and Elvehjem and Hart (*12*) found that ferric oxide gave negative results in the attempted cure of anemia in chicks.

In 1927 Doyle, Mathews, and Whiting (*10*) reported the results of 3 years' work on anemia in swine at the Purdue station. These investigators were unable to obtain the favorable effects of feeding iron to the sows that were obtained by McGowan. A possible explanation is that the pigs may not have consumed sufficient amounts of the sow's feed to include the necessary iron. In the Purdue experiments the iron was given in the form of ferrous lactate added to the mineral mixture to the extent of 1 percent, and the mineral mixture made up 2 percent of the ration. It may, therefore, be calculated that each little pig would have to consume daily well over one half pound of the sow's feed in order to get 25 mg of iron, a daily dose which has been successfully used by subsequent workers.

The Purdue workers found that anemia was about four times as prevalent, and the death rate nearly four times as high, among pigs kept indoors as among those kept outdoors on concrete. These workers concluded at this time that some factors in outdoor conditions were responsible for the prevention of the disease.

In the next year's work on anemia at Purdue different lots of sows and pigs were fed liver meal, wheat-germ meal, iron lactate, copper sulphate, manganese sulphate, and liver extracts in the rations. None of these feeds, minerals, or extracts were reported to have prevented anemia (*5*). In a further study (*33, Rpt. 42*) of possible environmental conditions pertaining to the development and prevention of anemia in pigs, groups of sows with their litters were (1) confined in a central hog house, (2) confined in a central hog house and exposed 30 to 35 hours per week to sunlight filtered through common glass, (3) the same except that the sunlight was filtered through Corex D glass, (4) exposed for 4 hours daily to rays from a carbon arc lamp, (5) confined outdoors in concrete-floored pens, and (6) kept in a pasture lot with a small individual hog house. The results of weekly hemoglobin determinations indicate no severe cases of anemia in any of these groups, although the pigs on pasture were the only ones which returned to and maintained a normal level of hemoglobin in the blood after the second week. The pigs irradiated with the carbon arc lamp and the pigs exposed to the sun's rays filtered through

different kinds of glass were little, if any, better off than the group confined indoors without irradiation of any kind. The pigs kept outdoors on concrete floors had a slightly higher hemoglobin content than had those retained indoors, but in this case their hemoglobin level was less than 50 percent normal at the end of 5 weeks. Mathews, Doyle, and Whiting (*29*) have further shown that exposure to the rays of a mercury-vapor-quartz lamp did not prevent anemia in pigs. The ineffectiveness of the direct rays of the sun or of ultraviolet-ray irradiation of the pigs to cure anemia has been further proved by the Wisconsin workers (*17, 18*). The results of 5 years' work on anemia in pigs at Purdue, where 120 litters numbering about 1,200 pigs were used, have been summarized recently by Craig (*7*).

The results obtained by the Purdue workers are quite similar to those obtained by Schofield (*32*) in Canada. According to this investigator, anemia frequently developed in suckling pigs during the first 2 weeks of life and, further, the feeding of iron salts, or iron and copper salts to the pregnant sows failed to prevent the disease in the pigs. Pigs raised outdoors, however, were resistant to the disease.

Hart and his associates at Wisconsin were probably the first to use copper salts as a supplement to iron salts in the cure of milk anemia in pigs. A report of the work done at that station appeared in 1929 (*17, 18*). A solution containing soluble iron and copper salts given to the pigs by mouth in amounts equivalent to 25 mg of iron and 5 mg of copper daily was highly effective in curing the disease. Commercial iron (ferric) sulphate solution alone was also highly effective; however, these workers attribute the beneficial effects of crude iron salts to copper contamination, without which, they maintain, iron would be ineffective for hemoglobin regeneration. Hart and his associates point out the fact that anemia is common in pigs only between the ages of 3 and 6 weeks. If pigs fail to contract the disease before they start eating solid feed, they rarely become anemic. Neither do late-farrowed pigs contract the disease when they have the opportunity to root in the soil and eat a small amount of green vegetation long before they ordinarily begin eating of the sows' feed. The explanation of these facts lie in the iron (and copper) present in the soil and in most feeds.

The spontaneous outbreak of the disease in a few litters in the experimental herds in the spring of 1929 at Illinois started some work on methods for prevention of the disease. Previous reports (*15, 20*) from this station have confirmed the results obtained by Hart and his associates. Dosing the little pigs with a solution containing iron and copper salts was highly effective in preventing and in curing the disease. Feeding iron and copper salts to the sow before and after farrowing apparently did not increase the content of these metals in the milk. Litters farrowed in a central farrowing house but removed to cindered pens within a few days after farrowing did not develop the disease. A convenient method of preventing the disease was found to be painting the udder of the sows once or twice daily with an aqueous solution of iron and copper salts to which had been added a little corn sirup.

The same beneficial effect of allowing the little pigs access to soil, pasture, or cinders out-of-doors has also been observed at Purdue by allowing the pigs access to blue-grass sod placed in the pen inside a central farrowing house (*33, Rpt. 43*).

Doyle, in still more recent publications (*8*, *9*), calls attention to the fact that soil alone, placed in the pens with pigs inside a central farrowing house, prevents the disease, although the hemoglobin content of the blood of these pigs is not so high as when blue-grass sod is used. Sows may be confined inside small houses, but if the little pigs gain access to an adjoining pasture, anemia will not develop. Green feed free from soil, when fed to the sow and pigs, failed to prevent anemia in the pigs, according to Doyle.

The simple and sure method, suggested by the Illinois workers (*15*, *20*) of preventing anemia in suckling pigs reared indoors, by painting the sows' udders with a solution containing iron and copper salts, has been successfully adopted by the Wisconsin (*6*), Kansas,[3] and Cornell (*27*, *Rpt. 43*) stations. It has been found at the Wisconsin station that brushing the sows' udders once daily is sufficient. Also, if the dosing method is used, a weekly dose of 150 mg of iron (ferric sulphate) and 25 mg of copper (copper sulphate) is effective and saves time and labor.

Willman and McCay (*27*, *Rpt. 44*) at Cornell, during a second year's study of practical methods of preventing anemia in pigs, found that drenching the pigs twice a week with a solution of ferrous sulphate (copperas) and then placing 0.1 percent of the salt in a palatable grain mixture, accessible to the little pigs after they were 14 days of age, entirely prevented the disease. They also reported that the blood hemoglobin was slightly higher when the pigs were drenched than was the case when the sow's udder was painted three times daily. Drenching with a copper-free iron salt was found just as efficient as was drenching with the copper-contaminated iron salts.

Studies of practical methods of prevention of anemia in pigs were continued at the Illinois station during 1930 and 1931. A summary of these results was presented by the senior writer to the twelfth annual Illinois veterinary conference, held at Champaign-Urbana, February 10, 11, and 12, 1931. In the following month, Hastings (*19*) expressed the views of a veterinarian on the subject of nutritional anemia in pigs. These ideas were so contrary to the findings at the Illinois station, as well as at other laboratories, that some comments on that article and also some of the results of the 1930 and 1931 studies at the Illinois station were later made by Carroll and Hamilton (*4*). The present paper describes in detail the later developments in the study of methods of preventing anemia in pigs, a brief summary of which has appeared in annual reports from this station (*21*, *23*).

EXPERIMENTAL RESULTS

During the spring of 1930, 62 litters of pigs were used in anemia investigations. The dates of farrowing ranged from early February till late April. All the pigs used were purebred from the Duroc-Jersey, Hampshire, Chester White, and Poland-China breeds. The sows varied in age, some were gilts and some were older sows. All pregnant sows were fed and cared for in accordance with good farm practice. All sows had been fed in dry lots. About a week before farrowing the sows were placed in the central farrowing house and kept in individual pens. The sows' rations were not supplemented

[3] AUBEL, C. E. Private communication.

with iron or copper salts. The litters were divided into five groups or lots,[4] the division being arbitrarily made by placing the first litter to farrow in lot 1, the second in lot 2, and so on.

All pigs were weighed individually at birth, at 4, and usually at 8 weeks of age. Records were kept of the apparent condition of all pigs, of all deaths prior to 8 weeks, and in addition, of the performance of all pigs from this investigation that were placed on subsequent feeding trials. In 17 litters blood samples and weights of individual pigs were taken at birth and at least weekly thereafter until the end of the period of observation, usually 8 weeks.

Blood samples were taken from the ears of the pigs. A flashlight held beneath the ear was found greatly to facilitate puncturing a blood vessel of proper size. Hemoglobin concentration was determined by the acid hematin method of Newcomer (*30*), the Newcomer disk being standardized by the Van Slyke oxygen-capacity method (*35*). All hemoglobin values are expressed as grams of hemoglobin per 100 cc of blood. Cell-volume[5] determinations were made by the use of hematocrit tubes of 1-mm bore. Heparin was used as an anticoagulant. The method used is similar to that described by Went and Drinker (*37*) in their micromethod for blood-volume determinations. As stated above the litters of pigs were divided among five different lots.

The little pigs in lot 1 received no mineral supplement. The sows and pigs were kept indoors throughout the period of observation, usually 8 weeks. Each litter was confined in a small pen 8 by 10 feet, with concrete floor. Over part of the concrete was a board floor, and straw was used for bedding. The little pigs in each litter had access to the mother's feed and feces, although excess feed was not allowed to remain in the troughs, and the floors were kept reasonably clean. On one side of each pen was woven wire fencing, and the troughs were of cast iron.

The pigs in lot 2 received no mineral supplement. Within a day or two after farrowing the sow and litter were removed to an outside house with board floor. The houses were well made and were supplied with plenty of straw for bedding. The houses were placed in a corner of a pen 8 by 16 feet, with concrete floor. The little pigs had access to the sow's feed and feces as in lot 1 but not to soil or vegetation. On warm days the sides of the houses facing the run were opened, and, whether they wanted it or not, the pigs were given a sun bath.

Lot 3 was confined indoors as described for lot 1. However, the udders of the sows in this group were brushed three times daily, from farrowing until the end of the period of observation, usually 8 weeks, with a solution containing iron and copper salts. The solution used contained approximately 25 mg of iron and 5 mg of copper per cubic centimeter and was made as follows: 19.64 g of hydrated copper sulphate and 89.51 g of anhydrous ferric sulphate were dissolved in 500 cc of water, and this mixture was then added to an equal volume of corn sirup.[6]

[4] Some slight modification of this plan was made in the case of a few litters, on which blood studies were not made because of the lack of space within the central farrowing house.

[5] By cell volume is meant the percentage of cells in whole blood.

[6] In certain litters in this group the treatment was modified as indicated in the figures. The composition of these modified supplements was as follows: The iron-sirup solution was the same as the iron-copper-sirup solution without the copper sulphate. The iron solution without copper and sirup contained 17 ounces of hydrated ferric sulphate in one half gallon of water.

Lot 4 was confined indoors as described for lot 1, and for the first 4 weeks after farrowing no supplement was available. From the fourth to the eighth week after farrowing the little pigs only had access to a palatable feed mixture which contained 0.1 percent of added crude ferric sulphate.

Lot 5 was also housed inside. During the first 4 weeks after farrowing the udders of the sows of this group were painted with the iron and copper solution as described for lot 3. From the fourth to the eighth week the udders of the sows were not painted, but the little pigs had access to a palatable feed mixture containing 0.1 percent of added ferric sulphate.

The results obtained are illustrated in figures 1 to 5, showing the trend from birth to the end of the period of observation, of the blood-hemoglobin concentration and the weights of individual pigs in litters from each of the five groups.

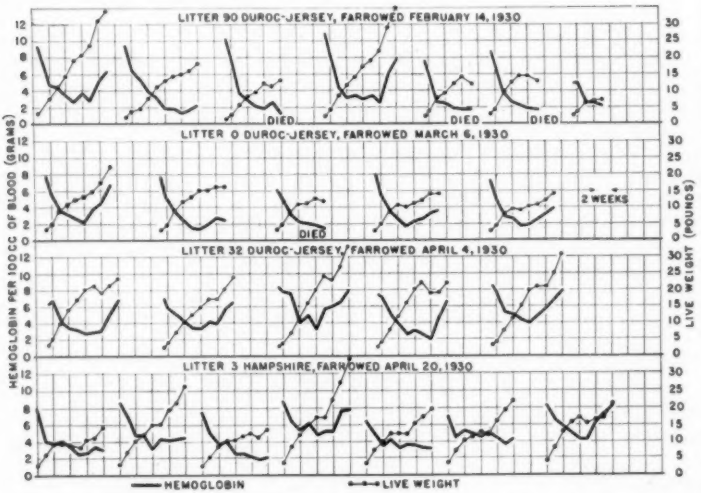
LITTER 90 DUROC-JERSEY, FARROWED FEBRUARY 14, 1930
LITTER 0 DUROC-JERSEY, FARROWED MARCH 6, 1930
LITTER 32 DUROC-JERSEY, FARROWED APRIL 4, 1930
LITTER 3 HAMPSHIRE, FARROWED APRIL 20, 1930
DIED
2 WEEKS
HEMOGLOBIN PER 100 CC OF BLOOD (GRAMS)
LIVE WEIGHT (POUNDS)
HEMOGLOBIN
LIVE WEIGHT

FIGURE 1.—Blood-hemoglobin concentration and live weights of pigs in lot 1. The animals were kept inside and received no mineral supplement.

Figure 1 gives the curves for the hemoglobin concentration and live weights of pigs in four litters in lot 1. These pigs were confined indoors and received no mineral supplement. The chart is self-explanatory, and from it the following conclusions are drawn: (1) Pigs kept indoors and subsisting solely on their mother's milk are more likely to become anemic if farrowed during February and March than they are if farrowed in April; (2) anemia is likely to be more severe in the February and March litters than in April litters; (3) the mortality of anemic pigs is greater in the February and March pigs than in the April pigs; and (4) putting off the farrowing date to April does not insure pigs against anemia, if they are confined indoors and allowed access to a diet of milk alone.

Figure 2 gives similar data for three litters of pigs in lot 2. These pigs were kept under outdoor conditions and received no mineral

supplements. This figure indicates that early farrowed (February) pigs may become severely anemic and that the mortality may be very great, even though they are housed out of doors. Although the pigs in this group did not have access to soil, it is doubtful if this would have made any difference in the behavior of the February litter, since the weather was such as to cause the little pigs to eat and then huddle close together in the straw most of the time. For the March and April litters, however, there seem to be some beneficial effects of outdoor conditions, although there was access to neither soil nor vegetation. Sunlight as a factor seems to have been ruled out by the Purdue and Wisconsin workers. In view of what is now known concerning this disease, it seems reasonable to suppose that the environmental conditions during the late spring are such as to induce the little pigs to root around and eat small amounts of the sow's feed and feces at an earlier date than is the case with the early farrowed pigs.

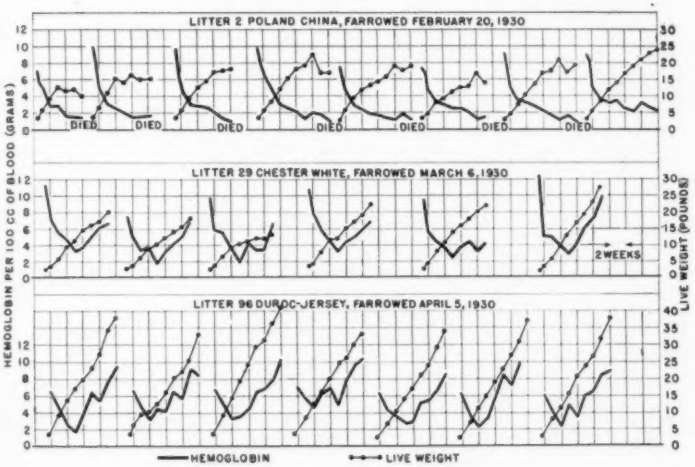
LITTER 2 POLAND CHINA, FARROWED FEBRUARY 20, 1930
DIED
LITTER 29 CHESTER WHITE, FARROWED MARCH 6, 1930
2 WEEKS
LITTER 96 DUROC-JERSEY, FARROWED APRIL 5, 1930
HEMOGLOBIN PER 100 CC OF BLOOD (GRAMS)
LIVE WEIGHT (POUNDS)
HEMOGLOBIN
LIVE WEIGHT

FIGURE 2.—Blood-hemoglobin concentration and live weights of pigs in lot 2. The animals were kept outside on concrete and received no mineral supplement.

The additional exercise induced also increases the little pigs' appetites perhaps beyond that satisfied by the milk supply, and this is another incentive to consume solid feed earlier. Along with the consumption of solid feed is the consumption of sufficient hemoglobin-forming essentials to enable the little pigs to escape the development of anemia.

Figure 3 gives the curves for the blood-hemoglobin concentrations and live weights for five litters in lot 3. This lot was kept indoors on concrete, but the milk diet of the little pigs was supplemented with iron, or iron and copper salts. The supplements were administered by painting the sows' udders with a solution containing these mineral salts. The first two litters, February and March litters, received the iron-copper-sirup supplement. Following the customary initial drop during the first few days, the hemoglobin concentration rose rapidly so that at the end of 2 weeks it had reached a nearly normal level.

Because of the possible toxic effects of copper sulphate, both to the little pigs and to the sow, and because it is questionable whether or not the little pigs as usually cared for need additional copper in their diets, data for two litters are presented to show the effects of the udder treatment when the copper is omitted from the solution. For the fifth litter, the concentration of iron was doubled and both the copper salt and the sirup omitted.

The data presented in figure 3 demonstrate the efficacy of iron and copper, or of iron salts alone, in preventing anemia in suckling pigs reared indoors. The udder treatment is shown to be a practical method of administering these mineral salts. That neither the sirup nor the copper salt is essential in the treatment is also indicated.

Figure 4 gives the blood-hemoglobin concentration and live weights of three litters of pigs in lot 4. In this group neither the sow nor the

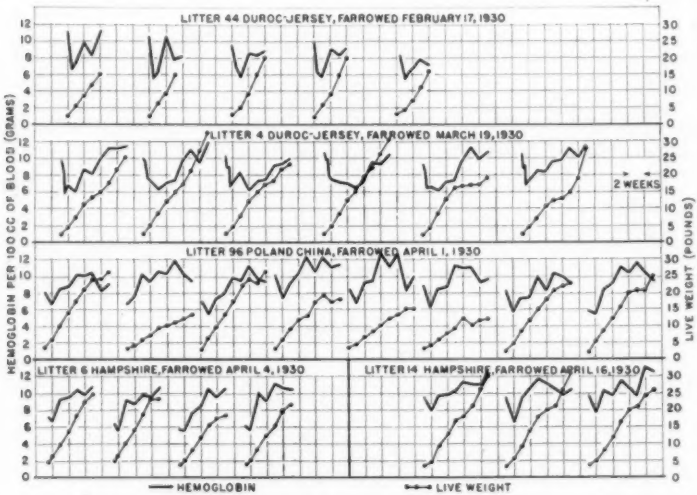
LITTER 44 DUROC-JERSEY, FARROWED FEBRUARY 17, 1930
LITTER 4 DUROC-JERSEY, FARROWED MARCH 19, 1930
2 WEEKS
LITTER 96 POLAND CHINA, FARROWED APRIL 1, 1930
LITTER 6 HAMPSHIRE, FARROWED APRIL 4, 1930
LITTER 14 HAMPSHIRE, FARROWED APRIL 16, 1930
HEMOGLOBIN PER 100 CC OF BLOOD (GRAMS)
LIVE WEIGHT (POUNDS)
HEMOGLOBIN
LIVE WEIGHT

FIGURE 3.—Blood-hemoglobin concentration and live weights of pigs in lot 3. The animals received iron and copper, or iron supplements. Litters 44 and 4 Duroc-Jersey received the iron-copper-sirup supplement throughout the period of observation; litters 96 Poland China and 6 Hampshire received an iron-sirup supplement, without copper; litter 14 Hampshire received an iron supplement only, without copper or sirup.

little pigs received any special treatment, other than their restricted confinement indoors on concrete, until the pigs were 4 weeks old, after which time the little pigs had access to a palatable feed mixture which contained 0.1 percent of added ferric sulphate.

Because of the unreliability of the treatment to which the pigs in this group were subjected, a more detailed description of the results obtained will be given. One litter was farrowed February 7. Twelve pigs were farrowed, but 1 died when it was 4 days old; the 11 pigs remaining all survived. At 17 days of age 9 of the 11 pigs were distinctly anemic, but at 59 days, when the test on this litter was discontinued, the average hemoglobin was 7.9 g. In contrast to the behavior of this litter is that of a litter farrowed 12 days later. This was also a large litter; 11 pigs were farrowed, but 1 died on the fourth day.

The average blood-hemoglobin concentration and live weight at birth were nearly identical for the two litters. While there were no deaths in the February 7 litter after the fourth day, there were five deaths, or 45 percent of the February 19 litter.

The third litter shown in figure 4 consisted of eight pigs farrowed March 20. Although the pigs in this litter had access to the iron-containing feed after they reached 4 weeks of age, they fared no better than the March litter in lot 1 which did not have access to the iron-rich feed. It may be noticed from the figure that the pigs in this litter all became severely anemic and that 5 of the 8 pigs died.

Figure 4 illustrates the fact that allowing the pigs access to an iron-containing supplement, which they may or may not consume, is of doubtful value. If the pigs have learned to eat solid food early enough (when 3 to 4 weeks old) the accessibility to an iron-rich supple-

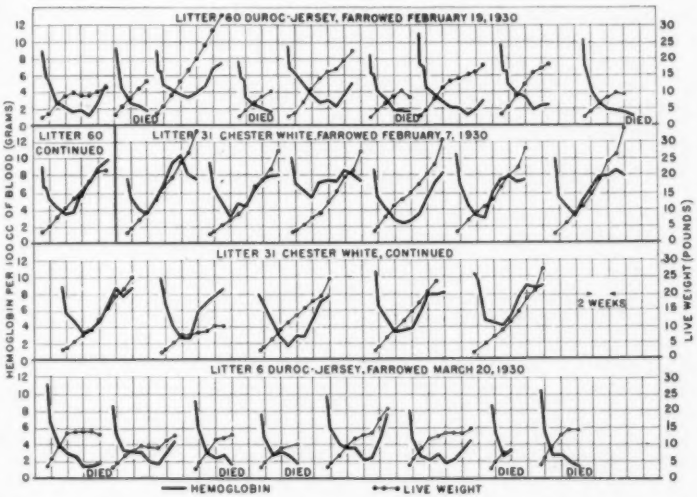
LITTER 60 DUROC-JERSEY, FARROWED FEBRUARY 19, 1930
DIED
LITTER 60 CONTINUED
LITTER 31 CHESTER WHITE, FARROWED FEBRUARY 7, 1930
LITTER 31 CHESTER WHITE, CONTINUED
2 WEEKS
LITTER 6 DUROC-JERSEY, FARROWED MARCH 20, 1930
HEMOGLOBIN PER 100 CC OF BLOOD (GRAMS)
LIVE WEIGHT (POUNDS)
HEMOGLOBIN
LIVE WEIGHT

FIGURE 4.—Blood-hemoglobin concentration and live weights of pigs in lot 4. The animals received no supplement the first 4 weeks, and then they had access only to feed to which 0.1 percent ferric sulphate had been added.

ment will serve to counteract the onset of anemia. Even without the iron-rich supplement such pigs eat the sow's feces and feed and obtain sufficient hemoglobin-forming essentials to prevent severe anemia. It was noticed, particularly in the case of the February 7 litter, that the little pigs began nibbling the sow's feed before they were 3 weeks old. It may have been that the sow, being unable to furnish sufficient milk to satisfy the appetites of such a large litter, was responsible for this.

In contrast to the behavior of the pigs which began eating solid feed at an early age is the behavior of the pigs in litters farrowed February 19 and March 20. But few of the pigs in these litters were noticed to be interested in solid feed by the time they were 4 weeks old. Most of these pigs were then so anemic (the average hemoglobin was 2.23 and 1.56 g, respectively, for these litters)

that, although an iron-rich feed was available, they were too weak and indifferent to consume it. Forty-five percent of the February 19 litter and 63 percent of the March 20 litter died, while none of the February 7 litter died after the eighth day. The average weight of the three pigs left in the March 20 litter at 53 days was only 15.5 pounds.

The treatment for the pigs in lot 5 consisted in painting the sows' udders with the iron and copper solution, from farrowing until the pigs were 4 weeks old, after which time the pigs had access to the feed containing 0.1 percent added ferric sulphate. The litters were kept indoors throughout the test. The usual data for two litters in this group are given in figure 5. The excellent condition of the pigs, as judged by the hemoglobin concentrations and live weights, is obvious.

In addition to the blood studies made on the spring pigs just described, 15 summer and fall litters were studied also. The pigs were confined either inside (lot 1) or outside (lot 2) on concrete and received no mineral supplement. In general the blood was examined only

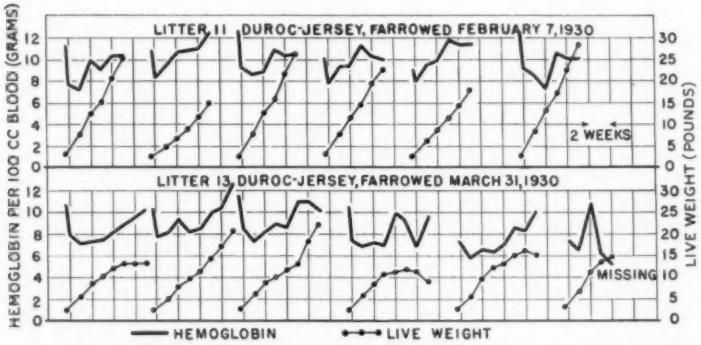

FIGURE 5.—Blood-hemoglobin concentration and live weights of pigs in lot 5. The animals received the iron-copper-sirup udder treatment during the first 4 weeks after farrowing; afterwards they had access only to a feed mixture containing 0.1 percent of added ferric sulphate.

at the end of the third and fifth weeks in order to determine the incidence and severity of the anemia developed under these conditions.

One litter of six pigs was farrowed July 6 and placed in lot 1 (inside, untreated). This litter failed to develop severe anemia. The average hemoglobin value at 22 days was 5.82 g and immediately thereafter showed an increase, so that at 36 days the average was 7.7 g.

Thirteen September litters were placed in the untreated lot 2 outside group. There were 71 pigs in these litters, and 14 (or 20 percent) had a hemoglobin concentration of 3.5 g or less at 3 weeks of age. Three litters containing 12 pigs were examined at 5 weeks of age, and the average hemoglobin concentration was 6.98 g. Only 1 of the 12 pigs was anemic at this time.

While several September litters showed an incidence of anemia of but 20 percent, late spring pigs or fall pigs may become severely anemic. This is illustrated by a litter of six pigs farrowed October 6, 1930, and confined on concrete under lot 2 (outside) conditions. The average hemoglobin concentration of the blood of this litter was, at 25 days, but 2.01 g. All six pigs were severely anemic, the maximum

hemoglobin concentration being 2.63 g. These pigs were immediately treated, and the test was discontinued.

In the spring of 1931, 3 litters, 1 farrowed March 25, 1 March 30, and 1 April 2, were used to study the effect of enforced exercise upon the incidence of anemia as determined by the blood hemoglobin. The procedure followed was to number each pig as soon after birth as possible and to divide each litter into approximately two equal groups, one group to be exercised and the other to be confined. In dividing each litter attempts were made to distribute the pigs according to sex, weight, and vigor. All litters were confined indoors,and on or be fore the third day the exercise was begun. During the night all pigs were allowed to remain with their mothers. During the day all pigs were confined in barrels with straw as bedding. At stated intervals during the day all pigs were placed with their mothers to feed for short periods of time. The groups to be exercised were removed from the barrels and driven up and down the concrete runway of the farrowing house twice daily. The runway was about 55 feet long, and the pigs were driven up and down this runway 7 to 14 times each exercise period. There were 10 exercised pigs, 7 of which became anemic; there were also 10 pigs not exercised, and 8 of these became anemic. The experiment was discontinued after 4 weeks, during which time there were 2 deaths in each group, apparently unassociated with anemia because the deaths occurred during the first week.

During this exercise test the little pigs had little, if any, chance to consume any feed other than the sow's milk. While the pigs were with the sows during the day for short intervals, they were closely watched to see that they did not root around in either the sow's feed or feces. It was only after dark that the pigs were allowed to stay with their mothers for any length of time, and it is doubtful that they stirred around much during this time. As an additional precaution, all metal parts of the pens were wrapped with burlap.

The blood-hemoglobin concentration and live-weight curves for these pigs are shown in figure 6. The results of this test indicate that exercise, in itself, is not a factor either in the development or in the prevention of anemia in pigs. It is quite possible, however, that exercise may, under normal conditions of management, increase the appetites of the pigs to an extent not satisfied by the milk supply of the sow, and in this case they take to solid feed quicker than would otherwise be the case. This, as well as any other factor which would induce the pigs to consume feed, feces, soil, etc., at an early age, would certainly tend to prevent the development of anemia.

The onset of anemia [7] in suckling pigs usually occurs between the third and fifth weeks, although anemia was often observed as early as the second week and may persist as long as the sixth week or even longer. The highest death rate occurs between the third and fifth weeks. Usually if the pigs live beyond the fifth week they have learned to eat solid feed and they recover rapidly. Average figures for live weights of pigs at 6 weeks of age seem to bear this out. The average live weight for all treated groups at 6 weeks of age was 19.5 pounds, and the average for the untreated pigs at the same age was also 19.5 pounds.

[7] In this investigation a pig is considered anemic when the blood-hemoglobin concentration reaches a value of 3.5 g or less per 100 cc of blood.

It has been estimated that approximately 35 percent of all pigs farrowed in the United States die before they reach the weaning age. That anemia may be an important factor in this connection, especially in early litters, is strongly indicated by the results of this study. In the following calculations concerning the incidence of anemia and death, all pigs that died or were killed before the eighth day were excluded inasmuch as their deaths were, in all probability, unrelated to milk anemia.[8]

The data indicate that among pigs restricted to a diet of milk only and confined either indoors or outdoors on board or concrete floors, anemia is not only more prevalent but also the mortality is greater during February and March than during the summer and fall. Of the pigs in lot 1, inside, nontreated group, 100 percent of the February pigs became anemic, and 57 percent died; 100 percent of the March

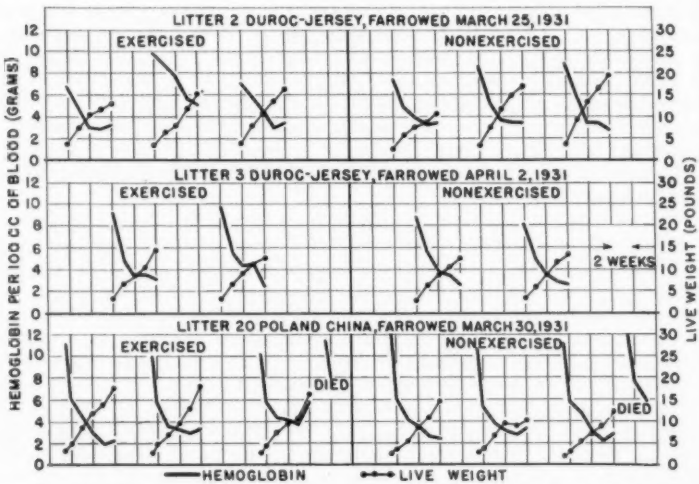

FIGURE 6.—Blood-hemoglobin concentration and live weights of exercised and unexercised pigs which received no mineral supplement.

pigs became anemic, and 20 percent died; 58 percent of the April pigs became anemic, but none died; and none of the July pigs became anemic and none died. Of the pigs in lot 2, outside, nontreated group, 100 percent of the February, 100 percent of the March, 86 percent of the April, 20 percent of the September, and 100 percent of the October pigs became anemic. The deaths in this group were 88 percent of the February pigs only.

A comparison of lots 1 and 2 indicates (1) that anemia may be just as prevalent and the mortality just as high in the outdoor litters as in the indoor litters in the very early months, (2) that anemia may be quite readily produced in litters restricted to board and concrete floors out doors any time of the year, and (3) that, although anemia

[8] As is customary in the university herd, pigs are vaccinated against cholera before they are weaned. All pigs in this investigation between the ages of 4 and 8 weeks were, therefore, on April 22, 1930, vaccinated with serum only and, on May 2, 1930, with the double treatment. Neither of these treatments seemed to affect the blood "picture" of the pigs, and there were no deaths following either treatment.

may be prevalent out doors on concrete, the mortality is not so great as indoors on concrete.

Grouping all treated pigs together, and all untreated (including lot 4) pigs together, it was found that of 157 pigs untreated, from February to October, 55 percent became anemic and 13 percent died, 24 percent of the anemic pigs died and none of the nonanemic pigs died. Of 38 treated pigs, farrowed between February and April, none became anemic. One pig in this group was missing. A closer examination of the untreated pigs shows that of 55 pigs farrowed in February and March, 96 percent became anemic and 38 percent died; 40 percent of the anemic pigs died, and none of the nonanemic pigs died.

As a practical aid in determining which of the several groups produced the largest numbers of satisfactory pigs, practically all the purebred litters of the 1930 spring season were placed, as previously described, in five groups and treated according to the procedure designated for that particular group. Some modifications in treatment were necessitated because of lack of barn space, and this modification amounted to the addition of two more groups, in one of which the udder treatment was followed by removal of the pigs to cindered lots not later than 2 weeks after farrowing, and in the other no treatment was given during the first 2 weeks and then the pigs were removed to cindered lots. The use of cindered pens was made because of the highly satisfactory results obtained during the previous year where this method was employed.

Sixty-two litters, a total of 301 pigs, were thus divided into 7 lots. The subsequent performance, especially in regard to the suitability for further feeding trials beginning in July and August, of all pigs available, was recorded. While practically all the pigs used in this investigation were eventually placed in feed-lot trials, the ones selected at this time were the best available. The selection of pigs for feed-lot trials was entirely unbiased, for after the 8 weeks of treatment all pigs were combined or mixed without regard to litters or previous groupings into convenient-sized pens for summer growth. It was not until after the feed-lot trials were started that the individual pigs were traced by their numbers to determine to which group in the anemia experiment they belonged. It was then found that 53 percent of the pigs in the treated groups were selected; 56 percent of the pigs which received no treatment but which were moved outside on cindered pens not later than 2 weeks of age were selected; while but 46 percent of the pigs that were untreated during all or the first 4 weeks of life were suitable at this time.

These data indicate that the treatments imposed to prevent anemia are in no way harmful to the subsequent health and well-being of the pig. Anemic pigs, although completely recovered from that condition, may be slower to regain complete health and proper appearance than pigs which have never had the disease. That subsequent growth of pigs once anemic may be interfered with is also indicated, although the average weights of pigs once anemic and of those which had never been anemic was found to be identical at 6 weeks of age.

As to the immediate cause of death of anemic pigs there is no direct evidence. It is perfectly obvious that as the anemic condition progresses the pig becomes weaker and weaker. Post-mortem examination shows a greatly enlarged heart—obviously nature's attempt to

send a greater volume of the weakened blood to the tissues. The pleural cavity is also filled with fluid so that the overworked heart is working against unusual pressures. The mere act of laying a severely anemic pig across one's knee for the taking of blood samples or of running him a short distance is sufficient to cause him to collapse. It seems most likely that the lessened vitality of the anemic pig makes him more susceptible to respiratory infections and other diseases which may be the immediate cause of death.

STATISTICAL STUDIES

Since the percentage of cell volume and the hemoglobin concentration were determined in samples of blood taken from pigs soon after birth and at least weekly for a period of several weeks, ample data were available for the study of the correlations between these two measurements as well as those between either of these measurements and the hemoglobin saturation of the cells, the latter measurements being readily calculated from the former two.

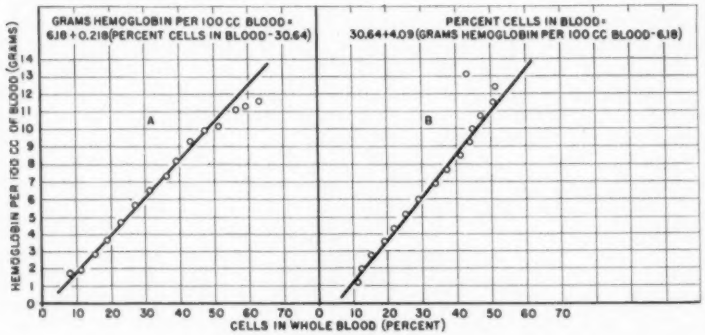
GRAMS HEMOGLOBIN PER 100 CC BLOOD = 6.18 + 0.218 (PERCENT CELLS IN BLOOD - 30.64)
PERCENT CELLS IN BLOOD = 30.64 + 4.09 (GRAMS HEMOGLOBIN PER 100 CC BLOOD - 6.18)
HEMOGLOBIN PER 100 CC OF BLOOD (GRAMS)
CELLS IN WHOLE BLOOD (PERCENT)
A
B

FIGURE 7.—Regressions of (*A*) hemoglobin concentration of blood on percentage of cells in the blood and (*B*) percentage of cells in the blood on the hemoglobin concentration of the blood.

It was obvious from the first that a high degree of correlation existed between the hemoglobin concentration and the cell volume in the blood of anemic pigs. Statistically this relationship, as expressed by the correlation coefficient r, was found to be $+0.945 \pm 0.0024$.

The regression equations calculated from these studies are:

(1) Percentage cell volume = 30.64 + 4.09 (hemoglobin concentration of blood − 6.18).

(2) Hemoglobin concentration of blood = 6.18 + 0.218 (percentage cell volume − 30.64).

(3) Hemoglobin concentration of cells = 19.52 + 0.705 (hemoglobin concentration of blood − 6.11).

(4) Hemoglobin concentration of blood = 6.11 + 0.521 (hemoglobin concentration of cells − 19.52).

(5) Percentage cell volume = 30.47 + 1.342 (hemoglobin concentration of cells − 19.54).

(6) Hemoglobin concentration of cells = 19.54 + 0.0965 (percentage cell volume − 30.47).

The regression lines are shown in figure 7. It may be readily seen that the plotted means of arrays cluster more closely around the regression lines below about 10 g of hemoglobin per 100 cc of blood than

they do above this value. This can hardly be explained on the basis of fewer data available in this upper region or of a greater biological variation when hemoglobin values exceed about 10 g per 100 cc of blood because of the fact that the means of arrays above this value all lie to one side of the regression line. The most probable explanation seems to be that, above a value of about 10 g of hemoglobin, there is little or no correlation between the blood-hemoglobin concentration and the cell volume. This would seem to indicate that above a certain hemoglobin concentration there may be an increase traceable to a hypersaturation of cells with little or no increase in cell volume.

When the hemoglobin concentration and the cell volume are known, it is a simple matter to calculate the hemoglobin concentration of the cells (expressed in grams of hemoglobin per 100 cc of cells). It was found that there was a significantly positive correlation between the hemoglobin concentration of the blood and the hemoglobin concentration of the cells. The correlation coefficient $r = +0.606 \pm 0.014$. This indicates that as the hemoglobin concentration of the blood decreases there is also a decrease in the hemoglobin saturation of the cells, and, since the erythrocytes make up such a large percentage of the cell volume, it means that there is a decrease in the saturation of the erythrocytes. This statement is supported by the calculations given in table 1. The figures in the last two columns of this table show that, as the blood-hemoglobin concentration decreases from 8.98 to 1 g per 100 cc, the concentration of hemoglobin in the erythrocytes decreases from about 14×10^{-12} g to about 11×10^{-12} g per erythrocyte. This decrease is small but consistent and is in harmony with the positive correlation mentioned above. It may be noted, however, that apparently there is no decrease in the mean corpuscular volume as the anemic condition becomes progressively worse. The mean corpuscular volume was found to be about $65\mu^3$. This is but slightly higher than the value of $57\mu^3$ reported by Götze (*14*) for the adult of the same species. The average hemoglobin content of the erythrocyte of adult swine blood has been reported by a number of workers (*11*, *14*, *28*, *36*), and the values given range from 17 to 22×10^{-12} g of hemoglobin per erythrocyte. These values are slightly higher than the value of 14×10^{-12} g reported here for the new-born pig.

TABLE 1.—*Size and hemoglobin content of erythrocytes at different stages of anemia*

Age or condition	Hemoglobin per 100 cc blood	Cell volume	Erythrocytes per mm^3 [a]	Erythrocyte		
				Volume	Hemoglobin content	
					$\times 10^{-12}$ gram [b]	$\times 10^{-12}$ gram [c]
	Grams	*Percent*	*Millions*	*Cubic microns*		
Less than 30 hours old	8.98	44.1	6.74	65	14.0	13.6
Slightly anemic	4.60	23	3.48	66	12.2	12.4
Moderately anemic	3.85	21	3.31	63	11.4	11.8
Severely anemic	1.7	16	2.47	65	10.62	11.73
Extremely anemic	1	10	1.5	67	10.60	11.70

[a] The number of erythrocytes in new-born pigs was taken from Hamilton, Hunt, Mitchell, and Carroll (*15*), while those values given for the anemic pigs were obtained in this study. The latter data were obtained on but few samples, so that they, as well as the calculations made from them, are to be considered more because of their relative rather than their absolute significance.

[b] Calculated from regression equation: hemoglobin concentration of cells = 19.52 + 0.705 (hemoglobin concentration of blood − 6.11).

[c] Calculated from regression equation: hemoglobin concentration of cells = 19.54 + 0.0965 (percent cell volume − 30.47).

A third correlation was possible in this connection, i.e., a correlation between hemoglobin concentration of the cells and the cell volume, and this was also significantly positive; $r = +0.360 \pm 0.020$.

It may be seen from the last two correlations that there is a closer correlation between hemoglobin saturation of the cells and blood-hemoglobin concentration than there is between hemoglobin saturation of cells and cell volume, the former correlation being, $r = +0.606 \pm 0.014$, and the latter, $r = +0.360 \pm 0.020$, the difference being 0.246, with a probable error of the difference of 0.024.

Thus the decrease in hemoglobin concentration of the blood, which occurs during the development of nutritional anemia, seems to be brought about not only by a decrease in cell volume but also, though not so important a factor, by a decrease in the hemoglobin concentration of the red cells.

A common opinion among practical swine raisers is that the incidence of anemia is greatest in litters that grow the most rapidly during the first few weeks. The data obtained in this study afforded an opportunity to test the validity of this opinion. The correlation between the decrease in blood-hemoglobin concentration occurring during the first 3 weeks and the increase in live weight during the same period was calculated for all untreated pigs in this investigation. Data were available for 72 pigs, and the correlation coefficient was found to be $+0.33 \pm 0.070$. Although not a high degree of correlation, it is nearly five times its probable error and may therefore be considered significant. The obvious explanation is that the more rapidly growing pig uses up his bodily store of minerals required for hemoglobin formation, which are present in insufficient concentrations in the milk, before it is old enough to consume solid food which contains the essential minerals, and therefore becomes more severely anemic than the slower growing pig.

THE NATURE OF THE INITIAL DECREASE IN BLOOD-HEMOGLOBIN CONCENTRATION AND CELL VOLUME

In previous publications from this laboratory (*15*, *20*) attention has been called to the fact that the concentration of hemoglobin in the blood of pigs begins to decrease at or very soon after birth. By determining daily for the first 5 days the hemoglobin concentration in the blood of a few litters of pigs treated with iron and copper, it was found that the hemoglobin concentration decreases continuously for 3 or 4 days regardless of the presence of an abundance of hemoglobin-forming constituents in the diet. However, if the milk diet is supplemented with iron or with iron and copper salts the hemoglobin concentration begins to increase about the fifth day and continues to increase so that by the end of the third or fourth week of life the birth level has been equaled or even surpassed. In the case of the pig the blood-hemoglobin concentration at birth seems to be about the same as the adult level.

In this investigation the hemoglobin concentration and cell volume were determined in blood samples taken as soon after birth as was possible, again in samples taken on the third or fourth day, and then at weekly intervals thereafter. Since some of the pigs were restricted to a diet of milk alone and others received an iron, or iron and copper, supplement with their milk, it was possible to study the magnitude

of the initial decrease, as measured by the hemoglobin concentration in the blood on the third or fourth day, in the presence and absence of the mineral supplements. The magnitude of the initial decrease in hemoglobin was determined by expressing the hemoglobin concentration on the third or fourth day as a percent of that found at birth of the pig. These values were averaged for the two groups, and for 61 untreated pigs the average was 68.5 ± 9.8, and for 28 pigs receiving the mineral supplement the average was 79.4 ± 6.8. While the difference between the means is 10.9 percent in favor of the pigs that received the mineral supplement the probable error of the difference is ± 12, showing the difference to be far from a significant one.

That the hemoglobin concentration in the blood of the treated pigs increases rapidly after the initial decrease and that it continues to decrease in rate on an unsupplemented milk diet is shown by the fact that the average hemoglobin concentration between the eighth and eleventh days for 16 treated pigs was 90.3 percent of their birth levels, while the corresponding average for 74 untreated pigs was 46.1 percent of their birth levels.

The cause of this initial decrease in blood-hemoglobin concentration is not explained by the data obtained in this investigation but, because of the consistency in the correlation between blood-hemoglobin concentration and cell volume in the blood from birth levels downward to severe anemic conditions, some speculation as to the nature of this initial decrease seems warranted.

The data presented above indicate that as the anemic condition develops in the pig, the hemoglobin concentration of the blood decreases not only because of a gradually decreasing number of red cells per unit of blood volume, but also because the hemoglobin concentration of the red cells also decreases. Insufficient data are available to show whether or not there is any change in the hemoglobin concentration of the cells coincident with the initial decrease in blood-hemoglobin concentration. Nevertheless it is certain that this initial decrease is caused mainly by a rapid decrease in the number of red cells per unit of blood volume.

A decrease in the number of red cells per unit of volume may be brought about either by a destruction of red cells more rapid than their formation or by an increase in blood volume in which the plasma is formed at a faster rate than are the red cells. It is conceivable that the formation of the blood cells during the first few days of postnatal life might be slower than it is after the animal has become acclimated to postnatal conditions, and it is also possible that there is some increase in red-cell destruction. That the latter may be a possibility of considerable importance may be inferred from studies made on the blood of new-born infants.

Rowntree, Brown, and Roth (*31*) have reviewed most of the work on the blood of the new-born infant prior to 1929. They conclude that the blood of infants at birth is extremely high in hemoglobin, often above 20 g per 100 cc of blood. More recent confirmation of this has been obtained by Börner (*2*) and by Elvehjem and Peterson (*13*). This high hemoglobin concentration is accompanied by a high cell volume. During the second week of the infant's life there is a decrease in hemoglobin concentration and cell volume, so that by the end of the second week these values have been reduced to those which

are considered normal for the adult. This decrease in hemoglobin concentration and cell volume is explained by Rowntree, Brown, and Roth (*31*) as being due to a hemolytic crisis which is probably related to icterus neonatorum. Whether or not this explanation applies to the initial decrease in hemoglobin concentration and cell volume that has been observed in the new-born pig, which has a hemoglobin concentration and cell volume equal to but no higher than that of the adult pig, remains to be shown.

SUMMARY

Nutritional or milk anemia in suckling pigs is more prevalent in February and March litters than in those farrowed in the late spring, summer, or fall. The disease is not only more prevalent during February and March, but it is also more severe and the death rate is greater. The disease, however, may be induced in litters farrowed during the late spring, summer, or fall by confinement of the litters indoors on board and concrete floors.

Anemia is just as prevalent in litters kept outdoors in individual houses with access to concrete runways as it is in litters confined indoors. Under outdoor conditions the mortality is somewhat less than under indoor conditions.

Brushing the udders of the sows with a dilute aqueous solution containing iron, copper, and sirup, or iron and sirup, or iron alone, one or more times daily from farrowing until weaning time, completely prevents anemia in suckling pigs. The udder treatment for the first 4 weeks followed by allowing the little pigs access to a palatable feed mixture containing 0.1 percent of added ferric sulphate is an alternative method which seems to be just as sufficient as is the udder treatment for the entire 8 weeks.

Of 55 pigs, farrowed during February and March and confined either indoors or outdoors on board and concrete floors without treatment, 96 percent became anemic and 40 percent of the anemic pigs died. Of 38 pigs, farrowed during February and March and confined indoors, that received either the iron and copper or iron treatment administered to the sows' udders, not one became anemic.

Nutritional or milk anemia is an important factor in the mortality of pigs before weaning time. Of 86 pigs having a blood-hemoglobin concentration of 3.5 g or less per 100 cc, 24 percent died. Of 109 pigs whose blood-hemoglobin concentration did not reach anemic levels, only 1 pig was lost; and whether or not this pig died, was eaten, or strayed away is not known.

Pigs receiving their mineral supplements through the udder treatment described above suffer no subsequent ill effects. A slightly greater percentage of treated pigs were suitable for feeding trials by July and August than was the case with untreated pigs.

Exercise in itself is not a factor in the prevention or the development of anemia.

The average hemoglobin concentration of 163 pigs less than 24 hours old was 8.98 g per 100 cc of blood, and the average cell volume of 98 pigs less than 24 hours old was 44.1 percent.

The correlation between the hemoglobin concentration in the blood and the cell volume is, $r = +0.945 \pm 0.0024$. The correlation between hemoglobin concentration in the blood and the hemoglobin saturation

of the cells is $r = +0.606 \pm 0.014$. The correlation between hemoglobin saturation of the cells and the cell volume is $r = +0.360 \pm 0.020$.

As the anemic condition becomes progressively worse, the blood-hemoglobin concentration, the cell volume, the number of cells, and the hemoglobin saturation of the cells decrease, while the corpuscular volume apparently does not change.

The mean corpuscular volume of the erythrocytes in new-born pig blood was found to be about $65\mu^3$ and the average hemoglobin content of an erythrocyte was 14×10^{-12} g.

Pigs which grow the fastest during the first 3 weeks are a little more likely to become anemic than are the more slowly growing ones. The correlation between the 3 weeks' increase in live weight and the 3 weeks' decrease in hemoglobin concentration was $r = +0.33 \pm 0.070$.

Pigs when farrowed have a hemoglobin concentration and a cell volume of about the same magnitude as those of the full-grown animal. During the first 3 or 4 days these values decrease to about two-thirds or three-fourths of the respective birth levels, regardless of the presence or absence of an abundant supply of hemoglobin-forming essentials in the diet. Possible causes of these initial decreases are suggested.

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ABSORPTION, UTILIZATION, AND RECOVERY OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY APPLE TREES GROWN IN CYLINDERS AND SUBJECTED TO DIFFERENTIAL TREATMENT WITH NUTRIENT SALTS[1]

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INTRODUCTION

In a recent paper the writer (*32*) [2] presented data on the composition of the season's branch growth at successive periods of the growth cycle in relation to the vegetative and reproductive responses of apple trees subjected to treatment with different combinations of nutrient salts, when grown in metal cylinders. The present paper reports the total amounts of nitrogen, phosphorus, and potassium (as N, P_2O_5, and K_2O) absorbed during the whole 6-year growth period by a representative tree under each treatment.

The trees in each of the two culture systems—sod and tillage—in this experiment were exposed to identical external conditions of temperature, rainfall, light, and heat, and in addition to a uniform soil substrate (*26*). Considered, therefore, as separate systems, the only variables to which the trees under sod and tillage, respectively, were subjected, therefore, were the mineral nutrient salts added.

A voluminous literature on the absorption and utilization of the principal nutrient elements by plants is extant. The writer (*27*, *28*, *29*, *30*, *31*) has critically discussed and summarized our present knowledge of this subject with reference especially to the absorption of nitrogen, phosphorus, and potassium, and Hoagland (*9*, *10*) has reviewed the more recent (1930–31) contributions. According to the views of Steward (*24*) and Hoagland (*9*) the process of absorption requires the expenditure of energy, the source of which is metabolic, determined by oxygen absorption or limited by carbon dioxide accumulation, the ultimate mechanism being determined by the electromotive forces brought about by oxidation-reduction potentials.

PREVIOUS INVESTIGATIONS ON THE TOTAL AMOUNTS OF NITROGEN, PHOSPHORUS, AND POTASSIUM REMOVED BY APPLE TREES

The object of the early experiments on the amounts of nitrogen, phosphorus, and potassium absorbed by the Pomaceae was to acquire information relating to the proximate amounts of these elements annually withdrawn from the soil by mature trees of maximum reproductive capacity.

Roberts (*20*) investigated a 13-year-old Wagener apple tree. The composition of the leaves sampled in the fall with respect to ash, nitrogen, phosphorus, and potassium was established. From these

[1] Received for publication Mar. 28, 1933; issued October, 1933. Technical Paper no. 597 of the Pennsylvania Agricultural Experiment Station. Presented before the joint sessions of the American Societies of Horticultural Science and Plant Physiologists at the Atlantic City meeting of the American Association for the Advancement of Science, 1932.

[2] Reference is made by number (italic) to Literature Cited, p. 579.

analyses and the average composition of apples obtained from another source, Roberts, by making numerous assumptions, presented an approximate estimate of the amounts of these nutrient elements removed by fruit and leaves at various ages of the tree.

Ten years later Van Slyke and his coworkers (*34*) reported the amounts of nitrogen, phosphorus, and potassium in the season's growth of a Baldwin and also of a Rhode Island Greening apple tree. The trees were about 30 years old. The approximate annual average removal of nutrients from the soil by trees of this age is reported as nitrogen, 1.5 pounds; phosphoric acid, 0.4 pounds; and potash, 1.6 pounds.

During the next decade Thompson (*33*) planted in the experimental plots of the Arkansas Agricultural Experiment Station seedlings obtained from five different known varieties of apple (average weight of trees 117 g). At the end of the growing season of each year the nitrogen, phosphorus, and potassium content of two trees of each variety were determined. Thompson reported the climatic conditions and also the analyses of the soil but did not mention either the fertilizer treatments (if any) or the culture system under which the trees were grown. Reference will be made later to certain of his data.

PLAN OF THE EXPERIMENT

Inasmuch as the plan of the present experiment has been given in detail in previous publications (*1*, *32*), it will only be necessary to recapitulate briefly. In May 1922, stocks representing a single clon were obtained from the East Malling Research Station, England, and were planted in a Hagerstown silty clay loam soil. Two weeks later they were whip-grafted to Stayman Winesap scions from a tree whose performance was known for two-bud generations (*1*). Boiler-plate cylinders 5 feet in diameter and 5½ feet deep were filled with the soil previously thoroughly mixed with respect to each of the three soil horizons (*26*, *32*). Three years after planting, the trees (six in each row) were subjected to differential nutrient treatments by the application of various combinations of the following pure salts: $NaNO_3$, $CaH_4(PO_4)_2.H_2O$, and K_2SO. The combinations added were (1) $NaNO_3 + CaH_4(PO_4)_2 + K_2SO_4$ (symbol NPK); (2) $NaNO_3 + CaH_4(PO_4)_2$ (symbol NP); (3) $NaNO_3 + K_2SO_4$ (symbol NK); (4) $CaH_4(PO_4)_2 + K_2SO_4$ (symbol PK); (5) $NaNO_3$ (symbol N); (6) $CaH_4(PO_4)_2$ (symbol P); (7) one row of trees received no nutrient salt addition (symbol check).

Half of the trees under each treatment were maintained in a timothy and bluegrass sod, and the other half were subjected to a tillage system. The practical field details and also the growth records during the experiment period under each system have been given by Anthony and Clarke (*1*). In October 1927, a representative tree growing under (1) the sod, and (2) the tillage systems, respectively, in each of the nutrient salt treatments was removed and divided for purposes of chemical analyses into (1) leaves, (2) trunk, (3) large branches, (4) small branches, (5) large roots, (6) small roots, and (7) fruit (if any).

Representative samples of the portions so separated were secured for analysis by first cutting them into small pieces, and then reducing by the process of successive quartering used in ore sampling. The

final samples representative of each division were dried at 100° F. and then ground in a Wiley mill to pass a 100-mesh sieve. Because of the difficulty in removing all the attached soil by washing, the results for the roots are calculated to a silica-free basis. The analytical methods adopted were identical with those previously described (*32*).

EXPERIMENTAL DATA

The analytical results are summarized in table 1. The detailed analyses are omitted, but investigators desiring the detailed analyses of the grafted Stayman Winesaps and of the trees when dug up and also of the prunings may procure them from the research office of the School of Agriculture of this institution or from the author. Photographs of six of the trees grown in sod are given in figures 1 and 2. Photographs of some of the other trees appear in the paper by Anthony and Clarke (*1*).

TABLE 1.—*Total nitrogen, phosphorus, and potash (grams) removed by trees from cultivated and uncultivated soil, to which different mineral treatments were applied, during entire growth period, 1922–27*

EXCLUDING LEAVES

Treatment	Dry weight of trees (grams)		Total N absorbed during growth		Total P_2O_5 absorbed during growth		Total K_2O absorbed during growth	
	Under cultivation	In sod	Under cultivation	In sod	Under cultivation	In sod	Under cultivation	In sod
Check	12,228	8,886	35.8	18.8	15.1	10.6	40.5	28.7
NPK	14,792	15,265	136.8	142.8	26.1	26.6	80.7	81.4
NP	16,005	15,607	129.6	138.2	23.1	25.2	71.4	65.3
NK	14,504	15,668	96.5	96.7	18.5	19.9	69.0	76.6
PK	8,996	8,326	39.8	28.6	13.0	13.4	41.5	33.8
N	12,109	13,053	83.3	84.0	15.3	16.4	51.1	56.6
P	12,239	7,595	53.4	31.2	17.1	11.5	50.1	29.5

INCLUDING LEAVES

Check	14,797	11,433	53.5	35.4	17.6	12.9	65.4	52.1
NPK	18,365	18,891	180.9	187.7	31.6	32.1	140.4	136.9
NP	19,277	18,975	170.3	180.3	27.8	29.9	109.3	99.6
NK	17,679	18,430	131.8	127.3	22.6	23.5	114.4	113.4
PK	11,788	11,054	63.4	55.0	15.8	17.3	79.7	71.1
N	15,419	16,139	121.3	119.0	19.9	20.7	90.9	93.0
P	14,936	9,907	77.1	47.9	20.4	14.0	79.8	54.9

INTERPRETATION AND DISCUSSION OF RESULTS

The distinguishing feature of the results is the great differences in the assimilation of nitrogen, phosphorus, and potassium resulting from the different nutrient salt treatments.

In considering the effect of these different treatments on carbohydrate metabolism, it has been found (*32*) that the concentration of carbohydrates in the season's growth follows the ascending series NPK < NP < NK < N < PK < P < check, showing that carbohydrates have accumulated in much larger amounts in the trees which did not receive nitrogen additions. The relatively small absolute amounts of nitrogen, phosphorus, and potassium found (table 1) in the trees receiving no nitrogen is an indication that the low concentrations of

FIGURE 1.—Trees grown in sod: *A*, no. E 1, treated with monocalcium phosphate only (symbol P); *B*, no. E 2, treated with sodium nitrate only (symbol N); *C*, no. E3, treated with sodium nitrate, monocalcium phosphate, and potassium sulphate (symbol NPK).

FIGURE 2.—Trees grown in sod: *A*, no. E 4, untreated check; *B*, no. E 5, treated with monocalcium phosphate and potassium sulphate (symbol PK); *C*, no. D 7, treated with sodium nitrate and monocalcium phosphate (symbol NP).

these elements calculated earlier (*32*) on the basis of percentage of dry weight of the season's branch growth is the result of a much lower intake of these elements and not a masking effect caused by the relatively high amounts of carbohydrates.

The concentration of nitrogen in the season's growth has been shown to follow the ascending series check <P<N<NK<PK< NPK (*32*). This order closely parallels the data for the total growth of the trees under each of the respective nutrient treatments. These results explain why the nitrogen factor plays such a dominant role in fruit growing.

SPECIFIC EFFECT OF EACH OF THE PRINCIPAL ELEMENTS

The writer has summarized (*28, 29, 31*) the results of field experiments which supply data on the effect produced, if one of the principal nutrient elements (nitrogen, phosphorus, or potassium) is omitted from a fertilizer. In some of these experiments (*13*) the omission of one of these elements resulted in an increased absorption of the other elements of the fertilizer with concomitant decrease in growth and yield of fruit attributed to a nutritional lack of balance. But in other field experiments (*23, 31*) the omission of an element from a fertilizer containing all three elements (nitrogen, phosphorus, and potassium) resulted in a decreased absorption of the other elements present. An explanation of such anomalous results has already been given by the writer (*31*).

The results summarized in table 1, which shows the amounts of nitrogen, phosphorus, and potassium (as N, P_2O_5 and K_2O) absorbed by the trees during the entire growth period of 6 years, indicate that the omission of one of these elements from a fertilizer has resulted in a decreased absorption and assimilation of the elements present in the incomplete fertilizer as compared with the complete fertilizer (cf. the amounts absorbed by the trees which received two nutrient elements, viz, NP, NK, and PK, with the absorption by the trees treated with all three nutrients, NPK). Such results are in accordance with Liebig's law of the minimum. This decreased absorption resulted in a lack of nutritional balance characterized by decreased growth and blossom formation except in the NP treatment, in which a decreased absorption of potassium resulted in slightly increased growth and blossoming.

In attempting to isolate the specific effect of an element in relation to the energy transformation in any living system, it is necessary to keep in mind the fact that the Wirkungswert (effect factor) of an element may not be (and generally is not) the same in the presence of another factor or factors as when the factor operates alone. Experimental results may be cited in which, when two factors vary simultaneously, each may produce its own effect independently of the other, as in Mitscherlich's experiments (*16, 22*). Mitscherlich found that when oats were grown in sand receiving varying amounts of water and calcium phosphate the relative effects on the yield produced by the different amounts of water were constant however much phosphate was added. But since numerous investigators (*5, 7, 14, 19*) have been unable to confirm the constancy of Mitscherlich's Wirkungswert for each fertilizer, the general principle enunciated in the opening of this paragraph must be accepted.

The data of the present experiment show that the amount of an added element absorbed by a plant and its effect on growth and reproduction is a function of the elements with which it is associated. The plant has a capacity to grow in a manner that cannot be regarded as a simple process of addition and subtraction of single effect factors. It is with these limitations in mind that the absorption and recovery of elements are discussed. An interesting and lucid explanation of the operation of growth factors is given by Nolte (*17*).

Effect of the Ommission of Nitrogen

Table 2 shows the amounts by which the absorption of each of the elements has been reduced by the omission of nitrogen and the quantitative effect of this reduced absorption in reducing growth and blossoming. The values given in columns 2 to 7 represent the difference in the amounts of the elements absorbed in each of two comparisons from which nitrogen has been omitted.

Table 2.—*Effect of the decreased absorption*[a] *of each of the nutrient mineral elements in reducing growth and blossoming following the omission of nitrogen, phosphorous, or potassium*

Treatments compared	N		P_2O_5		K_2O		Growth		Blossoms removed to thin to 6 inches apart, 1925–27	
	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod
NITROGEN OMITTED										
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Number*	*Number*
NPK and PK	117.6	132.7	15.7	14.7	60.6	55.7	6,577	7,836	530	727
NP and P	93.3	32.5	7.4	15.9	29.5	44.6	4,341	9,068	689	699
PHOSPHORUS OMITTED										
NPK and NK	49.2	60.4	8.9	8.6	25.9	23.5	686	461	272	348
NP and N	48.9	61.5	7.8	9.3	18.5	3.6	2,858	2,836	679	576
POTASSIUM OMITTED										
NPK and NP	10.7	7.3	3.8	3.2	31.0	37.3	[b] 911	[b] 84	[b] 195	28
NK and N	10.4	8.2	2.7	2.8	34.6	58.4	2,261	2,291	212	256

[a] Differences, in grams, in quantities of each element absorbed are shown in columns 2 to 7.
[b] In these cases only, an increase and not a decrease occurred.

The data in table 2 indicate that no simple quantitative relationship exists between the amounts by which the absorption of each of the elements have been reduced as a result of the omission of nitrogen from the fertilizer and the accompanying reduction in growth and blossoming. The effect values of nitrogen applied together with phosphorus are not the same when potassium is present as when that element is absent. Moreover, this effect changes from a negative to a positive value in the trees under cultivation and sod, respectively.

Effect of the Omission of Phosphorus

Table 2 also shows the amounts by which each of the elements has been reduced as a result of the omission of phosphorus from the fertilizer and the accompanying disturbance in metabolism shown by the extent to which growth and blossoming have decreased. The values given in the table express the difference in the amounts of the elements absorbed in each of two comparisons from which phosphorus has been omitted.

The table is of interest because it shows that the quantitative effect of the omission of phosphorus on the reduction of growth and blossoming is much greater in the absence of potassium than when this element is present. In other words, the effect factor for phosphorus when applied together with nitrogen is much greater if no potassium is added. Since the differences in the amounts of nitrogen absorbed under the respective culture systems in both comparisons are practically identical, one must attribute the marked effect of the omission of phosphorus in the present experiments to differences in the amounts of potassium absorbed.

Effect of the Omission of Potassium

Table 2 further shows the quantitative effect of the omission of potassium on the reduction in the absorption of each of the elements and the effect of this reduced absorption on growth and blossoming.

The omission of potassium resulted in increased growth in the combination NP, as compared with NPK, both under cultivation and in sod, and also in increased blossoming under cultivation. An explanation of this anomalous behavior will be apparent later from the discussion (p. 576) of the ratios in which the elements have been absorbed.

Utilization and Recovery of Nitrogen, Phosphorus, and Potassium

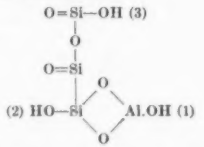
Theoretically a distinction must be drawn between the amount of an element absorbed and the amount utilized by the plant. Some authorities (*11*) claim that the absorbed but unutilized portion of a nutrient element is unchanged and is found as dissociated inorganic salts in the vessels and cell sap, and, although critical experiments on the subject have yet to be conducted, the fact that the results of many investigators (*2*, *23*, *35*) indicate that at least in some plants the portion of an element absorbed in excess is expelled via the roots shortly after blossoming time, would lend support to this view. If Jacob's views (*11*) are correct, absorption and the utilization of an element as determined by the analysis of a plant at maturity would be synonymous. Some of the results of this experiment, however, do not conform to this concept.

Present usage of the terms "absorption", "assimilation", "utilization", and "recovery" are somewhat confusing. In the present paper the term "recovery of an element" will be used to indicate that portion of an element applied as fertilizer which has been absorbed by the plant.

The amount of an element absorbed is determined, among other factors, not only by the species of plant and the availability of the fertilizer per se, but also by the indirect effects produced by the interaction of the fertilizer (or salt) with the components of the solid and liquid phases of the soil and also by interionic effects. These

effects, which may be very complex, are manifested in the so-called "phenomena of antagonism" and in the disturbance of equilibrium through displacement of bases in the soil colloidal complex by one or more of the elements in the fertilizer, and in the phenomenon of fixation by the iron and aluminum of the alumino-silicates as well as by the microorganisms—the so-called "biological absorption" (*25*). These effects were very marked in the present experiment.

In the clay soils of the type used in the present experiment the outer hydrophilic coatings of the clay particles dominate the soil system and, inasmuch as the majority of soils give rise to negative alumino-silicate ions, low results for the recovery by the plant of cations may in part be explained on the basis of adsorption. But the explanation of the low recovery of anions such as the phosphate ion by such a similar fixation is more difficult to explain, unless the explanation of some investigators (*12*) be accepted, of the existence of a Helmholtz triple layer, or of Clarens (*3*) of a clay structure represented by the constitutional formula



in which the basic hydroxyl group (*1*) attached to the aluminum ion can be replaced by acid radicles.

Recovery of Nitrogen

For reasons already given the recovery of an element from an added fertilizer cannot be determined with exactness in field experiments. In a controlled experiment of the present type (each cultural system considered separately), in which the trees have been subjected to the same growth factors except the addition of mineral salts, the values may be calculated accurately enough for practical purposes.

The values which represent the amounts of the respective elements absorbed from the added fertilizers given in tables 3, 4, and 6 were obtained from the difference between the amount of the element absorbed by the respective check tree and that of the treated tree under examination. This method gives values for the recovery somewhat higher than those that would be obtained by using instead of the check tree the tree which has received all the elements except the one of which the recovery is to be determined. The values for the recovery in the tables may thus be said to be upper limits.

In grain and vegetable crops the recovery of added nitrogen by the plant averages about 50 percent (*15, 18, 21*), but no data appear to be available on the recovery of nitrogen by fruit trees. In solution cultures at very high concentrations (0.02 to 0.16 percent) (*8*), the absorption-coefficient for the nitrate ion is very high, reaching 100 percent in the higher concentrations. The relatively low recovery by the plant of added nitrates in field experiments may be attributed not only to the low absorption-coefficient resulting from the relatively dilute concentration of the soil solution but also to the losses by leaching, to processes rendering the nitrogen immobile, and also (especially under cultivation) to losses as gaseous nitrogen.

The total amount of sodium nitrate applied to each of the trees receiving nitrate was 2,824 g, equivalent to 464.5 g calculated as elemental nitrogen. This corresponds to an annual application of 154.8 g, or to 0.33 pound of nitrogen per tree. The current practice in the experimental orchards of this station is to apply 5 pounds of sodium nitrate, equivalent to 0.8 pound of nitrogen, to mature trees, much larger than those used in the cylinders.

Table 3 shows the total nitrogen absorbed, the amount absorbed from the sodium nitrate added, and the percentage of the latter recovered by the plant.

TABLE 3.—*Total nitrogen absorbed by growing trees, and total grams and percentage of nitrogen absorbed from the added sodium nitrate*

Treatment	Trees under cultivation			Trees in sod		
	Total N absorbed	N absorbed from added $NaNO_3$	Added N utilized	Total N absorbed	N absorbed from added $NaNO_3$	Added N utilized
	Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
Check	53.5			35.4		
NPK	180.9	127.4	27.4	187.7	152.3	32.5
NP	170.2	116.7	25.1	180.3	144.9	31.2
NK	131.7	78.2	16.8	127.3	91.9	19.8
N	121.3	67.8	14.6	119.0	83.6	18.0

The utilization and recovery of added nitrogen follows the descending series NPK>NP>NK>N. The addition of monocalcium phosphate and potassium sulphate to the sodium nitrate either singly or together has, therefore, increased the efficiency of the latter. The data also show that the addition of monocalcium phosphate has increased the absorption of soil nitrogen, inasmuch as the increase in the amount of nitrogen absorbed by the trees treated with monocalcium phosphate compared with the check trees is 23.6 and 12.5 g in the cultivation and sod systems, respectively. These results may be compared with those of the Rothamsted wheat plots, in which the absorption of nitrogen by the crops on plots treated with potash and phosphates is very much greater than on the plots which received no such additions (*22*).

RECOVERY OF PHOSPHORUS

In high calcareous soils the addition of monocalcium phosphate results in the formation of insoluble di- and tri-calcium phosphates, the formation of the latter being greater the greater the $Ca:P_2O_5$ ratio. In the presence of magnesium carbonate, similarly difficultly soluble, calcium and magnesium phosphates are formed. Gerlach's experiments (*4*) may be given as an illustration of the high fixing capacities of calcium carbonate, magnesium carbonate, aluminum hydroxide, and ferric hydroxide for the phosphate ion. The phosphorus held by calcium carbonate and magnesium carbonate, however, is readily dissolved by a saturated solution of carbonic acid, whereas none of the phosphate fixed by the sesquioxides was dissolved.

In the absence of calcium carbonate, superphosphate is supposed to react with iron and aluminum compounds to form basic dicalcic aluminum and iron phosphates. This fact explains why the soil solution and the sap of plants contain so little phosphorus following

fertilization with phosphates. The concentration of phosphorus in the soil solution ranges between 1 and 2 parts per million. The drainage water of soils contains only a trace of phosphorus even in sandy soils (*6*).

The total amount of $CaH_4(PO_4)_2.H_2O$ supplied to each of the trees that received phosphorus was 1,869.0 g, equivalent to 1,052.5 g phosphorus, and corresponding to an annual application of 350.8 g or 0.77 pounds of phosphorus per tree. The current practice in the experimental orchards of this station is to apply 250 pounds of 16 percent superphosphate per acre of 100 trees, corresponding to 0.45 pounds of phosphorus per tree.

The total amount of phosphorus absorbed, the approximate amount of added phosphorus absorbed from the added phosphate, and the percentage utilized (recovered) is given in table 4.

TABLE 4.—*Total phosphorus absorbed by growing trees, and total and percentage of phosphorus absorbed from the added monocalcium phosphate*

Treatment	Trees under cultivation			Trees in sod		
	Total P_2O_5 absorbed	P_2O_5 absorbed from added $CaH_4(PO_4)_2$	Added P_2O_5 utilized	Total P_2O_5 absorbed	P_2O_5 absorbed from added $CaH_4(PO_4)_2$	Added P_2O_5 utilized
	Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
Check	17.6			12.8		
NPK	31.6	13.9	1.32	32.0	19.2	1.85
NP	27.7	10.1	.95	29.9	17.1	1.62
PK	15.8	−1.8		17.3	4.5	.42
P	20.3	2.6	.25	14.0	1.2	.12

The order of utilization and recovery of phosphorus in relation to treatment is NPK>NP>PK>P except in the PK treated trees under cultivation, in which a negative absorption of added phosphate occurred. The utilization of phosphorus and the recovery of added phosphates by all the trees are very low, the range being from 0.12 to 1.85 percent. This may be compared with the 6.0 percent average recovery of phosphorus from superphosphates in the Rothamsted experiments from field crops (*21*).

Table 5 shows that the total quantity of phosphorus in the soil used in this experiment is quite small. The sesquioxides are high (aluminum oxide and ferric oxide equal 13.94 percent in the surface soil and 19.16 percent in the subsurface soil), as is also the calcium oxide.

TABLE 5.—*Composition (percent) of the soil with respect to its principal basic and acidic constituents*

Constituent	Surface soil to 9.5 inches	Subsurface soil, 9.5 to 18.5 inches	Subsoil, 18.5 to 42 inches	Constituent	Surface soil to 9.5 inches	Subsurface soil, 9.5 to 18.5 inches	Subsoil, 18.5 to 42 inches
SiO_2	72.330	67.670	63.115	CaO	0.680	0.655	0.905
Al_2O_3	10.257	13.585	15.984	MgO	.665	1.015	1.360
Fe_2O_3	3.690	5.580	6.840	K_2O	3.925	4.200	4.410
P_2O_5	.098	.100	.077	Na_2O	2.260	1.290	1.315

These high values for aluminum and iron sesquioxides may account for the exceptionally small recovery of phosphorus by the plants in this experiment. It should, however, be emphasized that the mecha-

nism by which phosphorus is rendered immobile in the soil has not been definitely established, and no advances have been made since the writer reviewed the subject (*31*).

Recovery of Potassium

The total amount of potassium sulphate applied to each of the potash-treated trees was 1,027 g, equivalent to 548.4 g of potash. This corresponds to an annual application of 182.8 g, or to 0.40 pound per tree. Table 6 shows the total amount of potash absorbed, the approximate amount of potash absorbed from the potassium sulphate added, and the percentage of the latter recovered by the plant.

Table 6.—*Total potash absorbed by growing trees, total and percentage of potash absorbed from the added potassium sulphate*

Treatment	Trees under cultivation			Trees in sod		
	Total K_2O absorbed	K_2O absorbed from added K_2SO_4	Added K_2O utilized	Total K_2O absorbed	K_2O absorbed from added K_2SO_4	Added K_2O utilized
	Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
Check	65.4			52.1		
NPK	140.3	74.8	13.5	136.8	47.2	8.6
NK	114.4	49.0	8.9	113.3	61.2	11.1
PK	79.7	14.1	2.3	71.1	19.0	3.4

The order of utilization by the trees of added potash follows the descending series NPK > NK > PK. The amount of added potash recovered is relatively low. The range is from 2.3–13.5 percent. In field crops the average recovery of added potassium is 50 percent. The marked influence of sodium nitrate in combination with monocalcium phosphate and also of sodium nitrate applied singly in increasing the absorption of potassium is apparent. This effect may be attributed to the influence of the sodium and calcium ions of the added salts on the replaceable bases and also to other factors discussed by the writer (*31*).

Relative Proportions in Which Nitrogen, Phosphorus, and Potassium Have Been Utilized

The ratios of $N:P_2O_5:K_2O$ calculated on the basis of phosphoric acid as the unit, are shown in table 7.

In Thompson's experiments (*33*) the ratio for trees which had the same approximate dry-weight production but which apparently received no fertilizers is 3.9:1:3.4.

An unsolved problem of great practical significance to agronomists and horticulturists is the question as to whether each crop has its specific $N : P_2O_5 : K_2O$ ratio requirement, assuming that soil and climatic factors are constant. The results in table 7 lead to the conclusion that the ratios in which these elements are absorbed diverge from one another to a relatively small degree when plants that are optimum with respect to growth and reproduction are compared. In the present experiments, these are the trees which received the NPK and NP treatments.

TABLE 7.—*Variation in the ratio of nitrogen, phosphorus, and potash in the trees from cultivated and uncultivated soil to which different mineral treatments were applied*

EXCLUDING LEAVES

Treatment	Soil under cultivation			Soil in sod		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Check	2.37	1	2.68	1.76	1	2.69
NPK	5.24	1	3.09	5.37	1	3.08
NP	5.59	1	3.08	5.49	1	2.59
NK	5.20	1	3.72	4.85	1	3.85
PK	3.05	1	3.18	2.13	1	2.51
N	5.44	1	3.34	5.11	1	3.44
P	3.11	1	2.93	2.72	1	2.57

INCLUDING LEAVES

Check	3.03	1	3.71	2.74	1	4.05
NPK	5.72	1	4.44	5.85	1	4.27
NP	6.13	1	3.93	6.02	1	3.33
NK	5.82	1	5.05	5.42	1	4.83
PK	4.00	1	5.04	3.17	1	4.10
N	6.08	1	4.55	5.76	1	4.50
P	3.78	1	3.92	3.41	1	3.92

These results serve to explain the apparently anomalous behavior of the trees which received no potassium, referred to on page 572, in which a relative decrease in the absorption of nitrogen and phosphorus resulted in increased growth under both sod and tillage and in increased blossoming under cultivation. It would appear that there is a very delicate balance between the ratio of these dominant nutrient elements for optimum growth and that the concentration of potassium is too high relative to the nitrogen and phosphorus in the trees which received potassium additions. The potassium concentration exerts an enormous influence in determining the rate of diffusion of carbon dioxide into the cell and up to the chloroplast surface.

COMPARISON OF THE RATIOS OF $N:P_2O_5:K_2O$ ABSORBED BY TREES OPTIMUM WITH RESPECT TO GROWTH AND REPRODUCTION, WITH THE RATIOS IN WHICH THESE ELEMENTS HAVE BEEN APPLIED IN THE FERTILIZER ADDED

The method adopted in ascertaining the amounts of the respective elements which the trees under examination have absorbed from the respective salts added as fertilizer is not a mathematically rigorous one. This is a consequence of variation in the effect factors discussed on pages 570 and 572. The method of calculation is, however, sufficiently exact for all practical purposes. The very wide discrepancy between the ratio in which these elements are absorbed by the trees showing optimum physiological balance and the ratio in which the nutrient salts were applied is clearly brought out. The discrepancy is in part due to the soil properties, viz, leaching of nitrates and fixation of phosphorus and potassium.

After quantitative nature of these effects has been determined by an examination of the soil before and after the experiment, the theoretical economic quantities and ratios for optimum growth and reproduction of trees of this age will be given.

The total amounts of nitrogen, phosphoric acid, and potash added per tree were 464.5:1,052.5:548.4, equivalent approximately to a 3:8:4 fertilizer. In the trees analyzed the optimum ratio in which nitrogen, phosphoric acid, and potash have been absorbed is 175.6:29.7:124.8. By deducting from these values the amounts of nitrogen, phosphoric acid, and potash absorbed by the check trees, we get 122.1:12.1:59.5, or approximately 3.0:0.3:1.5 as the ratio in which these elements have been absorbed from the salts added compared with a 3:8:4 ratio actually applied.

An effort has been made and is still being made to remove fertilizer practice from the realm of empiricism. These results show the difficulties that must be overcome. The analysis of the plant alone is evidently insufficient. A knowledge of the fixing capacity of the soil is obviously of great importance. It follows that the application of the time-absorption-graph method on the entire plant (*31*) to the determination of the fertilizer requirements of the species when grown on soils of high fixing power necessitates a knowledge of the fixing capacity.

Although Wolff (*36*) provided definite proof that the composition of the ash of plants varied with the amounts and relative proportion of salts present in the nutrient culture or added to the soil, the present as well as the earlier results (*32*) cannot fail to emphasize anew the enormous differences that may be produced with respect to the percentage and absolute amounts as well as in the ratio of protein, carbohydrates, and ash constituents as the result of different fertilizer practices.

PRODUCTION OF DRY MATTER PER UNIT OF NITROGEN, PHOSPHORIC ACID, AND POTASH ABSORBED BY THE PLANT

Table 8 shows the amount of growth expressed in terms of dry weight of material formed per gram of the nutrient elements (as N, P_2O_5, and K_2O) absorbed by the trees in each treatment. The data exhibit another form of the operation of the law of diminishing returns inasmuch as the dry weights produced per unit of nutrient absorbed in the various treatments decrease as the total amount of that nutrient absorbed increases.

TABLE 10.—*Quantity of dry matter (grams) produced by the absorption of 1 gram of nutrient from cultivated and uncultivated soils to which different mineral treatments were applied*

Treatment	Grams of dry matter produced per gram of indicated nutrient element applied to soil					
	N		P_2O_5		K_2O	
	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod
Check	276	323	840	888	226	219
NPK	101	100	581	589	130	138
NP	113	105	694	634	176	190
NK	134	145	780	785	155	162
PK	186	200	745	637	148	155
N	127	135	773	781	170	173
P	194	207	733	706	187	180

SUMMARY AND CONCLUSIONS

In May 1922, Stayman Winesap apple trees propagated by whip-grafting scions from a single parent tree on roots vegetatively propagated from a single tree were planted in a Hagerstown clay loam soil contained in 5- by 5.5-foot cylinders. Each year from 1925 to 1927, inclusive, applications were made of the pure salts sodium nitrate, monocalcium phosphate, and potassium sulphate in different combinations. The annual application per tree was equivalent to 0.33 pound of nitrogen, 0.77 pound of phosphoric acid, and 0.40 pound of potash. With respect to cultural systems half the trees were in tillage and half in sod.

In October 1927, an entire tree representative of each nutrient treatment and also of each cultural system was removed, and the amounts of nitrogen, phosphorus, and potassium assimilated during the growth period of 6 years were determined.

The percentage and absolute (total) amounts of each of the elements absorbed by the trees during the whole period of growth and also the ratio in which they were absorbed varied greatly with the nutrient treatments and also, but to a much lesser extent, with the cultural system.

The values for the total amounts of nitrogen, phosphorus, and potassium absorbed by an entire tree in each of the respective treatments indicate that the omission of any one of these nutrient elements from the complete fertilizer (NPK) is followed by a decreased absorption of the remaining elements. This decreased absorption resulted in a nutritional lack of balance as exhibited in reduced growth and flowering except in one case in which potassium was omitted.

The Wirkungswert (the effect factor) for nitrogen, phosphorus, and potassium, varied with the elements with which the element considered was associated in the added fertilizers. These effect factors are discussed in detail.

The percentage recovery by the trees of added nitrogen, phosphorus, and potassium is relatively low under all treatments.

There appears to exist a specific ratio in which nitrogen, phosphorus, and potassium are absorbed by the trees (NPK and NP) which are optimum with respect to growth and reproduction. This ratio is approximately 6:1:4.

The wide discrepancy shown between the ratio in which these principal nutrient elements are absorbed by the trees—even by those showing optimum physiological balance (NPK and NP)—and the ratio in which the nutrient salts were applied is indicative of soil effects, namely, leaching of nitrates, and of high fixing capacities of phosphorus and potassium.

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A FACTOR IN THE VARIETAL RESISTANCE OF CRANBERRIES TO THE FALSE-BLOSSOM DISEASE [1]

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INTRODUCTION

Varieties of the cranberry (*Vaccinium macrocarpon* Ait.) under commercial culture differ widely in their susceptibility to the false-blossom disease. Fracker (*5*) [3] was probably the first to mention this fact in a publication; he states that in the inspection of Wisconsin bogs for this disease, in the course of regulatory work, a few varieties had "practically clear records". Stevens (*7*), summarizing present knowledge of varietal susceptibility, includes the Howes and Centennial among the sorts that are very susceptible to false blossom, and says that the Early Black shows a fair degree of resistance under varied field conditions and the McFarlin shows marked resistance. Beckwith (*2*) classifies the Centennial and Champion as "only slightly less susceptible" to false blossom than the Howes. The evidence upon which to judge the Champion is less conclusive than that relating to the other sorts; this variety, in New Jersey, is not so generally affected as either Howes or Centennial, but it has become badly diseased in some instances when exposed to heavy infection. On the basis of field observations it may therefore be said that the five varieties mentioned fall into the following descending order of susceptibility to false blossom: Howes and Centennial, Champion, Early Black, and McFarlin.

Varietal resistance to false blossom is almost certain to play a large part in the future of the cranberry industry, especially in the development of new commercial forms. Up to the present time the only method for judging the resistance of a variety has been to watch its behavior under exposure to natural infection for several years. It has not been known whether the resistance of McFarlin is due to natural protection against attacks of the insect vector [4] or to inhibition of the spread and survival of the false-blossom virus within the plant

[1] Received for publication Feb. 14, 1933; issued October 1933. Cooperative investigations of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the New Jersey Agricultural Experiment Station. Paper of the Journal Series, New Jersey Agricultural Experiment Station.

[2] The experiments reported herein were planned by the senior writer as one step in his attempt to devise a quick and reliable method of testing cranberry varieties for resistance to the false-blossom disease. They were conducted with the assistance of T. J. Blisard, formerly field assistant, New Jersey Agricultural Experiment Station, to whom acknowledgment is made.

[3] Reference is made by number (italic) to Literature Cited, p. 590.

[4] So far as known, false blossom is transmitted only by the blunt-nosed leaf hopper, as reported by Dobroscky (*3*). In the latest revision of the genus, Ball (*1*) gives this insect the name *Ophiola striatula* Fall. Common usage, however, still retains the name *Euscelis striatulus*.

tissues; or whether this variety may be a symptomless carrier of the disease.

There is no accumulation of evidence based on insect collections to indicate whether the blunt-nosed leaf hopper is relatively more abundant on Howes and Centennial bogs than on those of McFarlin or Early Black. In New Jersey, as a result of both natural conditions and cultural practices, such as the use of flood water for frost protection in June, the leaf-hopper population often varies greatly on plants of one variety, in a single bog, and even within the space of a few feet. Comparison between varieties in the field, therefore, is frequently misleading. A preliminary test showed little to be gained through casual observation of caged insects.

The present paper is a report of more careful experiments to determine whether the false-blossom vector prefers to feed on the "susceptible" varieties of cranberries rather than on those which are considered resistant; in other words, whether unattractiveness to the insect is a factor in the resistance of certain varieties. These experiments form part of an attempt by the senior writer to find a quicker and more reliable means of testing varietal resistance.

METHODS

Observation cages were prepared in the following manner: A 6-inch flowerpot was filled with moist sand and "planted" with 3 healthy uprights (cuttings) of each of the five varieties of cranberries heretofore mentioned, making 15 uprights in all. The pot was marked on the outside to identify the varieties. The uprights were enclosed in a lantern globe, the top of which was covered with cheesecloth. In this cage a number of mature leaf hoppers were placed. The interior of the cage could be examined from the outside without disturbing the insects. By keeping the sand moist, the uprights were maintained in good condition throughout the experiment (fig. 1).

On July 31, 1930, seven cages were prepared for the first series, the uprights for which were taken from bogs near Pemberton, N.J. About 25 leaf hoppers were introduced into each cage, and the cages were placed where they would receive direct sunlight only in the early morning and diffused light during the rest of the day. Observations were made in the afternoon. After the number of insects seen on cuttings of each variety had been recorded, each cage was turned so as to expose another variety to the strongest illumination for the next period. The experiment was continued until all the cages had been examined 10 or 11 times, making a total of 75 observations taken and 912 insect positions recorded.

A second series of 10 cages was prepared, the cranberry uprights for which represented the same varieties as before but were taken from bogs near Toms River, N.J. This series was not started until August 26, a very late date for work with this leaf hopper. The insect mortality was high, and fresh insects had to be added frequently. By this time, moreover, the cranberry fruit of the early varieties was very nearly mature. As it seemed possible that in the previous series the relatively large number of insects per cage might have interferred with their free choice of food plants; only five leaf hoppers were placed in each globe for the second trial. The cages

were kept just inside the screen of the laboratory porch at Toms River, where they received somewhat reduced sunlight during most of a clear day. They were examined once or twice daily until 18

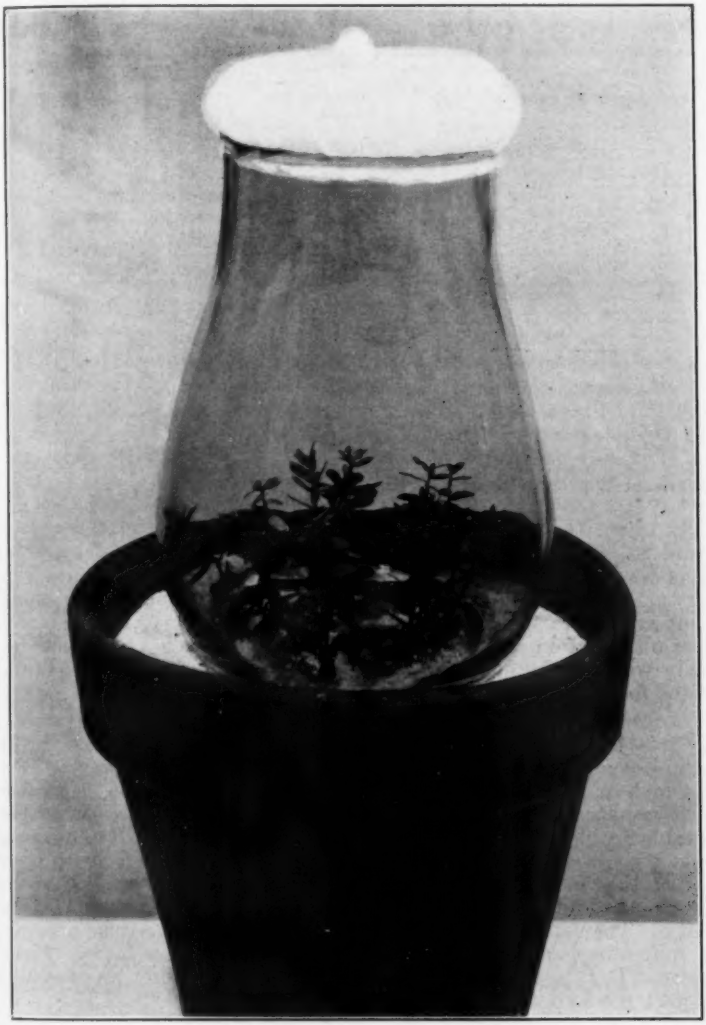

FIGURE 1.—Type of observation cage used in experiments on varietal resistance of cranberries to the false-blossom disease.

observations per cage had been recorded, or a total of 180 observations. The number of insect positions observed, however, was only 574, less than two thirds of the number obtained from the first series.

RESULTS

The results of the first experiment, as summarized by cages, are given in table 1. It is apparent that about twice as many insects were found on uprights of the Howes as on those of the McFarlin variety, that the proportion on Centennial was also high, and that Champion and Early Black were in an intermediate position.

TABLE 1.—*Leaf hoppers observed on uprights of 5 cranberry varieties in each of 7 cages*

[Data represent summations of 11 readings unless otherwise noted]

Cage no.	Leaf hoppers observed on uprights of—					
	Howes	Centennial	Champion	Early Black	McFarlin	All varieties
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1	32	33	42	29	17	153
2	36	35	20	25	26	142
3	30	48	22	39	19	158
4	36	24	24	28	16	128
5	37	23	23	24	4	111
6 [a]	36	30	22	15	19	122
7 [a]	27	20	23	10	18	98
Total	234	213	176	170	119	912
Percentage	25.7	23.4	19.3	18.6	13.0	100.0

[a] 10 readings.

In order to estimate the significance of these differences, the standard errors (standard deviations) were calculated. The variations were of the discontinuous type, and formed an asymmetrical curve. It was found by test that for each variety, and for the experiment as a whole, the frequencies with which 0, 1, 2, ... 9 insects were observed on a variety in a cage at one reading agreed satisfactorily with the frequencies calculated for a Poisson series according to the formula

$$e^{-m}\left(1, m, \frac{m^2}{2!}, \ldots\ldots \frac{m^9}{9!}\right),$$

in which m is the mean for the series, and 9! is "factorial 9".[5] The correspondence between observed and calculated frequencies is shown in table 2. The last line gives for each series the value of P as obtained from the table of χ^2 distribution.[6] This value represents the proportion of cases in which deviations from the calculated series will exceed the observed deviations. As agreement becomes closer, P approaches unity. The value $P = 0.05$ is regarded as the minimum to be accepted as indicating that the hypothesis accounts for the facts. Fisher states, "If P is between 0.1 and 0.9 there is certainly no reason to suspect the hypothesis tested."

[5] For a discussion of the Poisson series and its characteristics, and of the χ^2 method of testing goodness of fit, see Fisher (4).

[6] The value so obtained for the Early Black variety is somewhat too high; the necessary grouping of pairs for five or more insects in determining χ^2 disregards an obvious discrepancy in this case.

TABLE 2.—*Frequency of occurrence of various numbers of leaf hoppers on uprights of each of 5 varieties of cranberries, as observed and as expected according to Poisson distributions with similar means*

Number of leaf hoppers	Frequency of occurrence of leaf hoppers on uprights of—											
	Howes		Centennial		Champion		Early Black		McFarlin		All varieties	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
0	5	3.33	4	4.38	8	7.18	9	7.77	19	15.34	45	32.94
1	10	10.41	11	12.44	18	16.84	16	17.63	23	24.34	78	80.12
2	11	16.24	23	17.67	17	19.76	22	19.98	14	19.32	87	97.42
3	17	16.89	14	16.73	17	15.46	14	15.09	12	10.22	74	78.98
4	17	13.17	10	11.88	7	9.07	9	8.55	4	4.05	47	48.03
5	9	7.67	7	6.75	5	4.26	3	3.88	2	1.29	26	23.36
6	4	4.27	4	3.19	2	1.66	0	1.46	1	.34	11	9.47
7	2	1.91	1	1.30	0	.56	0	.47	0	.08	3	3.29
8	0	.74	1	.46	1	.16	1	.13	0	.02	3	1.00
9	0	.26	0	.15	0	.04	1	.03	0	------	1	.27
10 or more	0	.11	0	.05	0	.01	0	.01	0	------	0	.12
Total	75	75.00	75	75.00	75	75.00	75	75.00	75	75.00	375	375.00
P [a]	0.64		0.75		0.92		0.97		0.56		0.30	

[a] For explanation see p. 586.

The variance of a Poisson series is definitely preestablished. Since the observations for each variety agreed reasonably well with this formula, the standard error of random sampling for a variety (based on total insects rather than the mean) could be represented by the square root of the number of insects recorded on that variety in all observations. For example, the total for Howes would be $234 \pm \sqrt{234}$. The standard error of a difference between two varieties $(a-b)$ would then be $\sqrt{a+b}$; or, as an approximate figure to apply to a difference between *any two* of the five varieties, $\sqrt{\frac{2}{5}t}$, in which t is the total number of insect positions recorded on all varieties. Therefore, the very short formula used in these experiments for determining the standard error of a difference between the totals of two varieties was

$$\text{S.E.D.} = \sqrt{0.4t}.$$

This formula as applied to the first experiment gave a value of approximately 19, or 2.1 percent of the total number of insect positions. A difference exceeding twice this figure, or 38, was considered significant; a smaller difference might well be due to chance. In table 3 the differences between varieties are arranged in order of magnitude.

TABLE 3.—*Numerical differences between total number of insects found on uprights of 5 varieties of cranberries*

[Odds of probability calculated independently by Student's method]

Varieties		Difference in total number of leaf hoppers	Odds (Student's method)
More attractive to leaf hoppers	Less attractive to leaf hoppers		
Howes	McFarlin	115	1,924:1
Centennial	do	94	570:1
Howes	Early Black	64	78:1
Do	Champion	58	76:1
Champion	McFarlin	57	43:1
Early Black	do	51	25:1
Centennial	Early Black	43	72:1
Do	Champion	37	12:1
Howes	Centennial	21	6:1
Champion	Early Black	6	3:1

As a further test of significance the differences between all pairs of varieties were determined by Student's method from the values for each cage in table 1 taken as units. The odds determined from Love's modification of Student's table (*6*) were doubled, since, a priori, either variety of a pair might have been the more attractive.[7] These odds, which express the probability against the differences being due to chance alone, are given in the last column of table 3. Odds of 22:1, or greater, are considered significant.

It will be seen that the independent use of these two measures of significance gave the following identical indications: The differences between the attractive Howes and Centennial and the unattractive McFarlin were highly significant; there was a significant distinction between any two varieties separated by two or more places in table 1, i.e., between Howes and Early Black or between Centennial and McFarlin; whereas there were only insignificant differences between the adjacent Howes and Centennial, Centennial and Champion, and Champion and Early Black.

Table 4 gives the results of the second series of cages, each value being the summation of 18 readings. Detailed frequencies for this experiment are omitted, but they showed satisfactory agreement with Poisson series. The standard error of a difference was approximately 15, or 2.6 percent of the total number of insect positions. The only differences exceeding twice these values were between Howes on the one hand and the four other varieties on the other hand.

TABLE 4.—*Leaf hoppers observed on uprights of 5 cranberry varieties in each of 10 cages*

[Data represent summations of 18 readings (second series)]

Cage no.	Leaf hoppers observed on uprights of—					
	Howes	Centennial	Champion	Early Black	McFarlin	All varieties
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1	13	9	11	13	10	56
2	13	5	7	14	7	46
3	20	8	3	9	1	41
4	9	9	17	5	11	51
5	22	17	5	3	3	50
6	9	11	18	13	11	62
7	13	26	17	3	9	68
8	12	11	13	5	13	54
9	24	11	11	22	22	90
10	16	8	10	9	13	56
Total	151	115	112	96	100	574
Percentage	26.3	20.1	19.5	16.7	17.4	100.0

DISCUSSION

The significant differences in the second series corroborated the results of the first series. The variation in the behavior of varieties in the two series was apparently connected with the maturity of the early varieties by the end of August. The fruit of Early Black and Centennial was practically mature; it might be expected that the foliage of these varieties would also have become much less active

[7] For discussion see Fisher (*4*, *p.* 47).

than that of the late-maturing Howes and McFarlin,[8] and for that reason might have been less attractive to the leaf hoppers. It is interesting to note, therefore, that the percentage of insects on Centennial and Early Black dropped two or three points in the second series, and that these losses were absorbed by the relatively immature Howes and McFarlin, while the midseason Champion remained stationary. This behavior emphasizes the delicacy of the test and the fact that cuttings of equal vigor and stage of maturity must be used if results are to be regarded with confidence. So far as could be detected, the smaller number of leaf hoppers per cage in the second experiment did not influence the feeding preferences of these insects.

As previously stated, results of the first experiment indicate that the Howes and Centennial were definitely the most attractive to the leaf hoppers and that the McFarlin was the least attractive. The percentages in table 1, however, should be considered as relative rather than as quantitative measurements of the attractiveness of the varieties.

If attractiveness be regarded as susceptibility to insect attack, the Howes was the most susceptible; the Centennial, slightly less so; the Champion, midway; the Early Black, somewhat resistant; and the McFarlin the most resistant. These values and terms are in such complete agreement with the results of several years of field observation as to indicate a direct correlation between the degree of attractiveness of a variety to the insect vector and the rapidity and extent of the spread of false blossom in that variety. One can hardly escape the converse conclusion that the apparent resistance of certain varieties is due, at least in part, to their unattractiveness to the blunt-nosed leaf hopper. It is possible that the McFarlin or other varieties finally will be found to possess, in addition, some degree of actual resistance to the false-blossom virus.

The task of securing a wider selection of cranberries which are resistant to the false-blossom disease involves the testing of many varieties. The method used in these experiments gives a quick and reliable test of one type of resistance which appears to be of practical importance.

SUMMARY

On the basis of field observations extending over several years, the five varieties of cranberries used in the experiments have been arranged in the following descending order of susceptibility to the false-blossom disease: Howes and Centennial, Champion, Early Black, and McFarlin.

Observation of the insects in cages where they had free choice of food plants placed these varieties in the same order in regard to their attractiveness to the carrier of false blossom.

The attractiveness of cranberry varieties to the disease vector appears to be correlated with the rate of spread of the disease on these varieties under field conditions and, conversely, the apparent resistance of certain varieties to the disease appears to consist, at least in part, of resistance to the vector.

A convenient method is described for testing this type of varietal resistance.

[8] Experiments by Wilcox (*8*) indicate that changes occur in August which make even a late-maturing variety more resistant to false-blossom infection.

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FURTHER STUDIES OF SIZE AND SHAPE OF PLOT IN RELATION TO FIELD EXPERIMENTS WITH SUGAR BEETS [1]

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INTRODUCTION

The literature relating to standardization of field experiments with sugar beets (*Beta vulgaris* L.) was reviewed briefly by the senior writer in a previous paper.[2] While the literature dealing with the question of the most efficient size and shape of plots for testing varieties of some crops is very extensive, little has been published on the proper size of plots to be used in sugar-beet agronomy. So far as the writers are aware, the work reported in the paper just cited was the first extensive study of the relative efficiency of plots of varying size and shape in agronomic trials with sugar beets. The present paper presents further data on the same problem, as well as on the relation of stand to yields and the use of the regression of yield on stand as a means of estimating the error of the experiment.

MATERIALS AND METHODS

The experimental design of this study was essentially the same as that previously employed.[3] [4] A field of sugar beets, 110 by 330 feet, planted to the Kleinwanzleben Normal brand or variety in 1931, was chosen for the experiment. The field had been cropped in a uniform manner in previous years and cultural practices were the same over the entire area in 1931. On May 13 the beets were planted in rows 22 inches apart. At thinning time the beets were mechanically cross-blocked and then thinned to a single beet per 12 inches of row. At harvest time the field was marked out into plots 2 rods (33 feet) long. The field then contained 600 single-row plots 2 rods long and was 60 rows wide by 330 feet long, each row being subdivided into 33-foot units. All beets adjacent to skips in the rows were removed at harvest time and weighed separately for each plot. The remaining beets, namely, those with normal competition on all sides, were then harvested, counted, cleaned, and weighed. The average weight per root of these normally competitive beets in each unit plot was determined and then multiplied by 33 to obtain the theoretical yield of the plot on the basis of a perfect stand. The stand prior to removal of plants adjacent to skips was 83.3 percent perfect. After removal of all beets adjacent to skips the stand of normally competitive beets was 62.8 percent.

[1] Received for publication, July 11, 1933; issued, October 1933. Contribution from the U.S. Department of Agriculture in cooperation with the Minnesota Agricultural Experiment Station.

[2] IMMER, F. R. A STUDY OF SAMPLING TECHNIC WITH SUGAR BEETS. Jour. Agr. Research 44: 633–647. 1932.

[3] IMMER, F. R. (See footnote 2.)

[4] ——— SIZE AND SHAPE OF PLOT IN RELATION TO FIELD EXPERIMENTS WITH SUGAR BEETS. Jour. Agr. Research 44: 649–668, illus. 1932.

The analysis of variance [5] method was used in analyzing the data. The statistical technic employed has been discussed thoroughly in previous papers.[6] [7]

EXPERIMENTAL RESULTS

ANALYSIS OF YIELD DATA

The analysis of variance of yield was made on the assumption of five varieties or treatments to be tested. The experimental design is one of randomized blocks. The different sizes and shapes of plots studied were the same as were used before.[7] In table 1 is given the analysis of variance for weight of roots per plot of one row 2 rods long, expressed on the perfect-stand basis.

TABLE 1.—*Analysis of variance of weight of beets in single-row plots 2 rods long*

Variation	Degrees of freedom	Sum of squares	Mean square [a]	Standard deviation	z [b]
Between blocks	119	7,803.8239	65.5784	8.0980	0.3604
Within blocks	480	15,310.1400	31.8961	5.6477	
Total	599	23,113.9639			

[a] Mean square or variance $(S.D.^2) = \frac{\text{Sum of squares}}{\text{Degrees of freedom}}$.

[b] z = one half the difference between the natural logarithms of the 2 variances.

Since there were 600 unit plots in the test there would be 599 degrees of freedom for the total. The 120 blocks of 5 hypothetical varieties each would contribute 119 degrees of freedom, leaving 480 degrees of freedom for variation within blocks. The value of z, used in testing the significance of the difference between variances between and within blocks exceeded the 1 percent point, and it is concluded that variation in weight between blocks was greater than that within blocks. A significant reduction in the error (variation within blocks) has resulted, therefore, by removing the block effect. The mean square for variation of a single plot within blocks (31.8961) was greater than the mean square calculated in the previous study [8] in 1930 (21.9744). The average stand of normally competitive beets was 85 percent in 1930 and 63 percent in 1931. The variance (mean square) within blocks increased very nearly proportionately to the inverse ratio of the stand. The mean square between plots within blocks, as pointed out previously,[8] is made up of two components, (1) variation due to inherent soil differences between plots and (2) the number of beets per plot actually harvested.

In a similar way the variance of 3-row plots 2 rods long may be calculated when only the central row of each plot was considered harvested. By so doing it would be possible in an actual experiment to correct for differential competition between adjacent varieties by removing the border rows. The analysis of variance is given in table 2.

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London. 1930.

[6] IMMER, F. R. (See footnote 2.)

[7] ——— (See footnote 4.)

[8] IMMER, F. R. (See footnote 2.)

TABLE 2.—*Analysis of variance of weight of beets in 3-row plots 2 rods long of which only the central row was harvested*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	z
Between blocks	39	1,601.8942	41.0742	6.4089	0.0699
Within blocks	160	5,713.7480	35.7109	5.9759	
Total	199	7,315.6422			

There are now only 200 plots (of 3 rows each), which will give 199 total degrees of freedom. Since each block of 5 hypothetical varieties will require 15 single rows, there will be 40 such blocks, giving 39 degrees of freedom and leaving 160 for variation within blocks.

The variance within blocks (35.7109) is again higher than in the previous study [9] made in 1930 (23.5453), due partly to the reduced stand in 1931. The value of z indicates that the variation between blocks was not significantly greater than the variation within blocks, since the observed value of z (0.0699) did not exceed the 5 percent point.

Considering hypothetical plots 1, 2, 3, 4, 6, and 12 rows wide and 2, 4, 10, and 20 rods long, similar analyses of variance were made and the variances between the 5 plots within blocks determined. For plots 3 or more rows wide a single border row could be discarded on either side of the plot and the analysis of variance made on this basis. That differential competition does exist between adjacent rows of two different varieties of beets under some conditions and must be eliminated has been demonstrated.[10] In table 3 are given the standard deviations in percentage of the mean for these combinations of width and length of plot for 1931 and the average of 1930 and 1931. The average standard deviations were obtained by averaging the variances (mean squares) and extracting the square root.

TABLE 3.—*Standard deviation, in percentage of the mean, of yields of plots varying in size and shape in 1930 and 1931*

ENTIRE PLOT HARVESTED

Length of plot (rods)	Standard deviation of yields (percent) for plots of indicated width (rows)											
	1931						Average of 1930 and 1931					
	1	2	3	4	6	12	1	2	3	4	6	12
2	9.52	7.21	5.90	5.56	5.53	4.56	9.34	7.06	5.95	5.92	5.94	4.81
4	7.08	5.49	4.03	4.10	4.44	3.34	7.25	5.64	4.76	4.83	5.14	4.41
10	5.36	4.36	3.27	3.26	3.81	2.73	5.58	4.54	3.89	3.66	4.58	3.34
20	4.23	3.40	2.56	2.51	3.38	2.49	4.57	3.79	3.12	3.68	4.21	2.89
CENTRAL ROWS HARVESTED												
2			10.17	7.21	6.39	4.79			9.85	7.70	6.88	4.99
4			7.35	5.28	5.37	3.55			7.76	5.38	5.95	4.15
10			6.02	4.28	4.65	2.84			6.11	4.50	5.46	3.51
20			4.87	3.07	4.12	2.59			5.30	3.75	5.01	3.32

[9] IMMER, F. R. (See footnote 4.)

[10] Unpublished data.

As was to be expected, the standard deviation in general decreased as the size of plot was increased. When the entire plot was harvested in 1931 the error decreased more rapidly by increasing the length of the plot from 2 to 4 rods than by increasing the width. There seems to have been little difference between the effect of increasing the length from 10 rods to 20 rods and that of doubling the width. When the central rows alone were harvested, increasing the width of the plot reduced the standard error more rapidly than increasing the length of the plot.

When the entire plot was harvested, the average standard deviations for 1930 and 1931 showed very little difference between the efficiency of increasing the length of the plot and that of increasing the width as a means of reducing the standard deviation. When only the central rows were harvested, increasing the width of the plot was, in general, decidedly the more efficient method.

The efficiency of plots of varying size and shape was calculated by comparing the variances, per unit area, of plots differing in size and shape. A single-unit-plot basis for the study was secured by multiplying the variances per plot by the number of units (single rows 2 rods long) in each plot. Accepting the variance within blocks of single plots 2 rods long as a standard, the efficiency of plots of other sizes and shapes was determined by dividing the variances of a single-row plot 2 rods long by the variances of plots of other sizes and shapes, calculated on a unit basis. For example, in 1930 and 1931 the average variance within blocks, in percentage of the mean, for two-row plots 2 rods long, was 49.8268. The variance within blocks, for single-row plots 2 rods long, was 87.2699. To place the study on a unit basis, the variance of the 2-row plots was multiplied by 2, giving 99.6536. By dividing 87.2699 by 99.6536, 87.6 percent was obtained as the efficiency of the 2-row plots compared with the single-row plots, both being 2 rods long. In a similar manner the efficiencies of other combinations were determined. Table 4 shows the efficiency of these plots in 1931 and the average efficiency in 1930 and 1931.

TABLE 4.—*Percentage efficiency in use of land of plots varying in size and shape in 1930 and 1931*

ENTIRE PLOT HARVESTED

Length of plot (rods)	Percentage efficiency of plot of indicated width (rows)											
	1931						Average of 1930 and 1931					
	1	2	3	4	6	12	1	2	3	4	6	12
2	100.0	87.2	86.8	73.4	49.5	36.4	100.0	87.6	82.2	62.2	41.2	31.4
4	90.4	75.1	92.9	67.5	38.3	33.8	83.0	68.5	64.3	46.8	27.6	31.2
10	63.1	47.7	56.7	42.6	20.8	20.2	56.1	42.3	38.5	32.6	13.9	13.0
20	50.7	39.2	46.0	35.8	13.2	12.2	41.8	30.4	29.8	16.1	8.2	8.7

CENTRAL ROWS HARVESTED

2	.....	.....	29.2	43.5	37.0	32.9	.....	.....	30.0	36.8	30.7	29.2
4	.....	.....	28.0	40.6	26.2	30.0	.....	.....	24.2	37.6	20.5	21.1
10	.....	.....	16.7	24.8	14.0	18.7	.....	.....	15.6	21.5	9.8	11.8
20	.....	.....	12.7	24.1	8.9	11.3	.....	.....	10.4	15.5	5.8	6.6

When the entire plot was harvested the efficiency decreased as the size of plot was increased. The standard error decreased as the plot size was increased (table 3). This decrease was not proportional to the increased area of the plots, however, but resulted in a lower efficiency for the larger plots. It would be more advantageous, therefore, to use small plots and replicate sufficiently to reduce the error to the level desired, than to use larger plots and fewer replications. By so doing the total area of land required for the test would be kept at the minimum.

When only the central rows were harvested, the 4-row plots were decidedly the most efficient. When border rows are removed, more land is required for 3-, 6-, or 12-row plots than for 4-row plots in order to reduce the standard errors to the same level; therefore there would be little or no advantage in using the 3-row in preference to the 4-row width of plot. It is likewise questionable whether the slight decrease in the cost of planting and harvesting a smaller number of 6- and 12-row plots as compared with a larger number of 4-row plots would compensate for the increased cost of caring for the greater area of

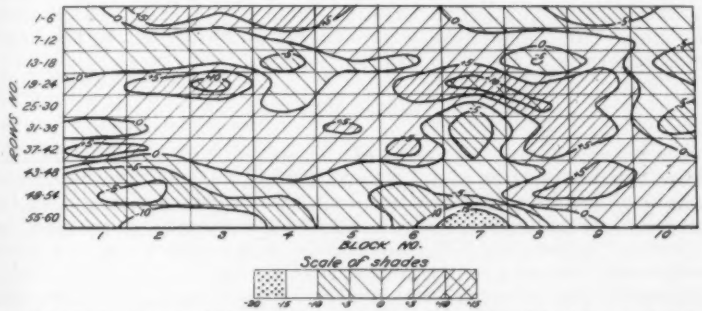

FIGURE 1.—Contour map of weight of roots from one hundred 6-row plots each 2 rods long; contour lines drawn through the points deviating by −15, −10, −5, 0, +5, and +10 percent from the mean weight.

land needed for the wider plots. When border rows are removed the 4-row plot seems to be the ideal width. Apparently it makes little difference whether these are 2 or 4 rods long. The latter length might be preferable, especially since poor stands are sometimes encountered.

In figure 1 is shown a contour map of soil heterogeneity on the field from which the following data were obtained. The average yield per row was determined for a field of one hundred 6-row plots each 2 rods long. Assuming this average yield to be found at the middle of each plot, the points where the yields were average, where they were 5 and 10 percent above the mean, and where they were 5, 10, and 15 percent below the mean were obtained by linear interpolation. Points where the yields showed similar deviations were joined by continuous lines, and the contour map shown in figure 1 was thus constructed.

The systematic nature of soil heterogeneity, as it influenced yield, is apparent. Toward the left side of the diagram the contour lines tended to parallel the rows to a very pronounced degree. Had the direction of these contours been known prior to planting time it would have been more advantageous to plant the seed in rows at right

angles to the direction of the yield contours. Toward the right side of the diagram the contours no longer paralleled the direction of planting to the same degree and it would have made little difference in which direction the rows were planted. The soil on this field appeared very uniform in type. It would have been impossible to predict the location or direction of these contour lines prior to the analysis of the yield data. In spite of the apparent uniformity of the soil type, differences as great as 20 percent can be found in the inherent yielding capacity of the soil in areas only 65 feet apart. The need of replication under such conditions is apparent. Considering the systematic nature of the soil heterogeneity the necessity of randomizing the arrangement of plots in a field trial is obvious also.

REGRESSION OF YIELD ON STAND

Knowing the total number of beets in a plot and the weight of these beets, it is possible to calculate the expected weight from the number of roots. It was assumed in this study that five varieties were to be tested in 4-row plots each. The regression of yield on stand was calculated within such blocks of 20 rows, each 2 rods long. The regression equation, expressing the expected weight of all beets in a single plot 2 rods long, within blocks of 20 rows each, the stand being known, was found to be

$$W' = 50.7022 + 1.566210\ (r' - \bar{r}'),$$

where W' is the expected weight of all beets and r' is the total number of beets in a single-row plot. The regression coefficient within blocks was calculated from the variance for root number and covariance for number of roots multiplied by weight within blocks, as discussed in a previous paper [11] [12]. The covariance within blocks is calculated in the same manner as variances, that is, by subtracting the sum of products due to blocks from the total sum of products, leaving the sum of products within blocks. In table 5 the significance of the regression coefficient (1.566210) is tested.

In testing the significance of the linear regression the observed value of z greatly exceeded the 1-percent point, and it is concluded that the regression undoubtedly was significant. The observed value of z found in testing the significance of the quadratic regression also exceeded the 1-percent point, and it is concluded that the quadratic regression

$$W' = 50.7022 + 2.503668\ (r' - \bar{r}') - 0.017923\ (r'^2 - \bar{r}'^2)$$

was significant also. Variation due to quadratic regression minus linear regression may be compared with variation due to deviations from quadratic regression in order to determine whether the quadratic relationship supplied more information than did the linear.

The value of z for this comparison (0.5962) did not reach the 5-percent point, and it may be concluded that the quadratic regression was not significantly better than the linear. The stands varied from about 50 percent (only 2 of the 600 plots had less than a 50-percent

[11] IMMER, F. R. (See footnote 2.)
[12] ——— (See footnote 4.)

stand) to 100 percent. Within this range the regression of total weight of all beets on the total number of roots was essentially linear.

TABLE 5.—*Tests of significance of linear and quadratic regression of weight of all beets on total number of beets and of increased accuracy of quadratic over linear regression*

LINEAR REGRESSION

Variation due to—	Degrees of freedom	Sum of squares	Mean square	z
Linear regression	1	14, 215. 5929	14, 215. 5929	3. 2945
Deviations from linear regression	569	11, 126. 4411	19. 5544	
Total within blocks	570	25, 342. 0340	44. 4597	

QUADRATIC REGRESSION

Quadratic regression	2	14, 279. 7662	7, 139. 8831	2. 9521
Deviations from quadratic regression	568	11, 062. 2678	19. 4758	
Total within blocks	570	25, 342. 0340	44. 4597	

QUADRATIC AND LINEAR REGRESSION COMPARED

Quadratic minus linear regression	1	64. 1733	64. 1733	. 5962
Deviations from quadratic regression	568	11, 062. 2678	19. 4758	
Deviations from linear regression	569	11, 126. 4411	19. 5544	

In agronomic trials beets adjacent to skips in the row are usually removed before harvest, and only plants with normal competition on all sides are harvested. The yield is then expressed on the basis of a perfect stand on the assumption that the average weight per plant harvested is the weight which would have been secured if a perfect stand had been obtained. The regression of the weight of competitive beets on the number of normally competitive beets (within blocks 20 rows wide and 2 rods long) was found to be given by

$$W = 37.2497 + 1.736746\ (r - \bar{r}),$$

where W is the predicted weight of the competitive beets and r is the number of normally competitive beets per single-row plot. In table 6 is given the test of significance of the regression coefficient.

TABLE 6.—*Test of significance of linear regression, within blocks, of weight of normally competitive beets on number of normally competitive beets in single-row plots 2 rods long*

Variation due to—	Degrees of freedom	Sum of squares	Mean square	z
Linear regression	1	34, 935. 0753	34, 935. 0753	3. 9548
Deviations from regression	569	7, 297. 6757	12. 8254	
Total within blocks	570	42, 232. 7510	74. 0925	

The observed value of z exceeded the 1-percent point, and it is concluded that the regression coefficient was significant. The mean square due to deviations from regression within blocks may be used as an estimate of the variance due to error, where "error" means deviation of the observed yields from the expected yields estimated from the regression relationship.

Another possible comparison presents itself, that obtained by multiplying the yield of all beets per plot, irrespective of skips, by a factor to convert the yield of each plot to a perfect-stand basis, as is done for competitive beets. After so raising the yield per plot, irrespective of skips, to a perfect-stand basis the error may be calculated in the usual manner. Since the regression of yield on stand was linear, such a practice would be as correct as raising the yields of competitive beets to a perfect-stand basis. The variance within blocks was found to be 31.5636. This is slightly lower than the variance calculated for perfect stands of competitive beets, but not significantly so. The slight increase in precision may be accounted for by the fact that the number of beets available was greater when all beets were taken than when only competitive beets were harvested. The slight deviation from linear regression was not great enough to offset the greater number of beets available when no correction was made for skips.

SUMMARY

Further studies are reported on size and shape of plot in relation to field experiments with sugar beets.

Standard errors, in percentage of the mean, decreased as the size of plots was increased.

When the entire plot was harvested, efficiency in the use of the land decreased as the size of plots was increased. When a single border row was removed on each side of the plot, the 4-row plot was decidedly the most efficient.

The regression of yield of all plants in a plot, irrespective of skips, on the total number of beets was essentially linear.

EFFECT OF EXCESS CARBON DIOXIDE ON GROWING MUSHROOMS [1]

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INTRODUCTION

In mushroom culture the problem of ventilation has always been considered important, but so far as the writer is aware there has been no experimental work done on it. It is a common observation among growers employing underground caves that mushrooms have a tendency to "sulk" in certain pockets in the caves. It has also been known for many years that they have a tendency to grow abnormally long stems and small caps when confined in a limited atmosphere under a glass jar or similar container.

A consideration of these observations led the writer to make a series of experiments in which cultivated mushrooms (*Agaricus campestris* L.) of the white and cream varieties were grown in a modified atmosphere. Systematic observations were also made on the carbon dioxide content of commercial mushroom houses and caves under different conditions in order to determine the practical significance of the results of these experiments.

METHODS

The atmosphere was modified by slowly running carbon dioxide, nitrogen, and oxygen in different amounts and combinations into glass jars inverted over clumps of mushrooms growing on a standard mushroom bed. In some cases the gases were introduced into the compost beneath the clumps of mushrooms rather than into containers above the bed. The gases were obtained in standard commercial cylinders, and the flow was regulated by means of reducing valves, pinchcocks, and bubbling bottles. The arrangement of the apparatus is shown in figure 1. The only gases measured were carbon dioxide and oxygen. With the Orsat gas analysis apparatus used in these experiments the smallest portion of gas that it was feasible to determine was approximately 0.2 percent. Judging from the determination of replicate samples, the error inherent in the use of this apparatus is not more than 0.1 percent.

The effect of different treatments on the growth of the mushrooms was determined by weighing and making outline drawings of the four largest mushrooms in treated clumps and comparing these with similar data from untreated clumps having apparently an equal chance at the start.

It was necessary to exercise considerable care in selecting comparable clumps of mushrooms. Most of the experiments were made at temperatures approximating 15° C.

[1] Received for publication July 3, 1933; issued October 1933.

Except where otherwise specified, the atmosphere surrounding the clumps of untreated mushrooms, which were used as checks, contained less than 0.2 percent carbon dioxide and approximately 20.8 percent oxygen. In the interstices of a normal section of the compost under an actively growing clump of mushrooms there was approximately 20 percent oxygen, and the carbon dioxide content ranged from 0.3 to 0.4 percent.

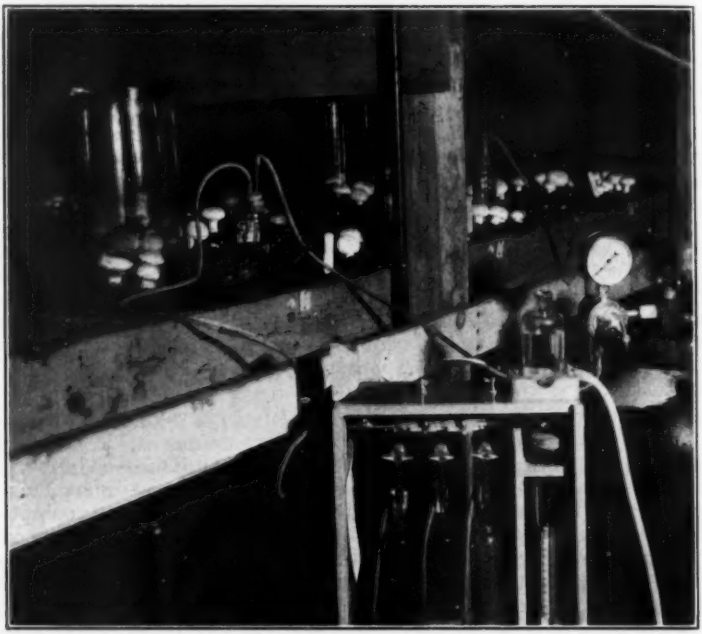

FIGURE 1.—Apparatus used for altering and testing the carbon dioxide and oxygen content of air surrounding growing mushrooms.

RESULTS OF EXPERIMENTS

EXPERIMENTAL SERIES NO. 1

The first group of experiments indicates that mushroom growth is arrested by diluting the atmosphere surrounding the caps and the mycelium with carbon dioxide, but there is no evidence to indicate whether the stunting is due to high carbon dioxide concentration or to the dilution of oxygen caused by the addition of carbon dioxide, nor is it clear whether the stunting is due primarily to the modification of the atmosphere surrounding the sporophores above ground or the mycelium in the compost.

EXPERIMENT 1-A.—Carbon dioxide was run slowly into a jar over a clump of button mushrooms for 5 days. The concentration of carbon dioxide varied from 6 to 18 percent, and that of oxygen from 16 to 19 percent. In the atmosphere of the compost beneath this jar the carbon dioxide content was 4.6 percent and the oxygen content 20.4

percent. As a result of this treatment the growth of the button mushrooms was completely arrested, while similar clumps outside of the jar

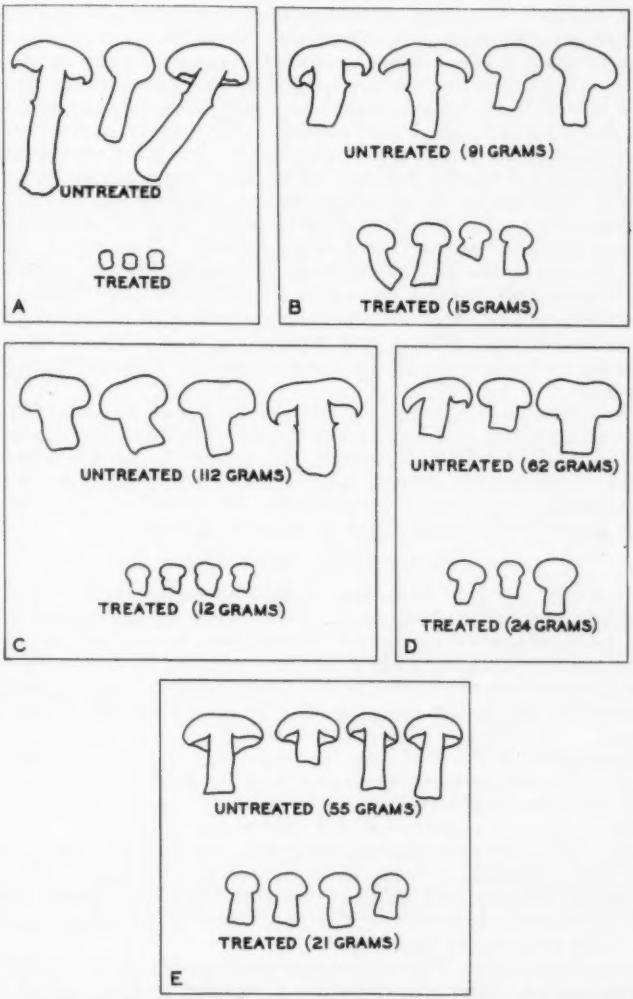

FIGURE 2.—Effect on the growth of mushroom sporophores of diluting the atmosphere with high concentrations of carbon dioxide. Treated mushrooms in group *A* were subject to 6 to 18 percent carbon dioxide for 5 days; group *B*, carbon dioxide 7.2 to 9.4 percent; *C*, carbon dioxide 8 to 9.6 percent; *D*, carbon dioxide 10.8 to 20 percent; *E*, carbon dioxide 15.6 to 22.4 percent. Untreated mushrooms in all had a comparatively equal start but were only subject to normal carbon dioxide in house, less than 0.1 percent.

grew to maturity. Figure 2, *A*, shows the comparative sizes of treated and untreated mushrooms at the end of 5 days.

EXPERIMENT 1-B.—Carbon dioxide was run into a jar over button mushrooms more slowly than in experiment 1-A. The concentrations of carbon dioxide in the atmosphere under the jar ranged from 7.2 to 9.4 percent; the range of the oxygen concentration was 19.0 to 20.2 percent. Growth was arrested, as shown in figure 2, *B*.

EXPERIMENT 1-C.—Carbon dioxide was run into a jar over button mushrooms, as in experiment 1-B. The carbon dioxide concentration of the atmosphere ranged from 8.0 to 9.6 percent, and the oxygen value ranged from 19.0 to 20.4 percent. The carbon dioxide content of the atmosphere in the compost beneath the clump of mushrooms ranged from 3.8 to 4.0 percent, while the oxygen remained at 20.2 percent. The results of this treatment are shown in figure 2, *C*.

EXPERIMENT 1-D.—A comparatively rapid flow of carbon dioxide was run into a jar over a clump of mushrooms. The carbon dioxide concentration in the jar ranged from 10.8 to 20.0 percent, while the oxygen was so diluted that it ranged from 16.8 to 18.4 percent. The atmosphere of the compost beneath the jar contained 6.0 to 6.6 percent carbon dioxide and 19.0 to 19.6 percent oxygen. The effect of this treatment on the growth of the mushrooms under the jar is shown in figure 2, *D*.

EXPERIMENT 1-E.—A comparatively rapid flow of carbon dioxide was passed into the jar. The carbon dioxide content of the atmosphere in the jar was 15.6 to 22.4 percent, the weighted average being 18.4 percent. The oxygen content ranged from 15.0 to 17.6 percent and the weighted average was 16.6 percent. The effect of this treatment on the growth of the mushrooms is shown in figure 2, *E*.

EXPERIMENTAL SERIES NO. 2

The second group of experiments shows that moderate dilution of the atmosphere with nitrogen has little effect on the growth of the mushrooms, whereas dilution with carbon dioxide even in the presence of an excess of oxygen causes a distinct stunting of the mushrooms. It would seem from these results that the increase in the concentration of carbon dioxide rather than the dilution of oxygen is the important factor.

EXPERIMENT 2-A.—Nitrogen was run into the jar to dilute the oxygen. The oxygen content ranged from 7.8 to 20.0 percent, and the carbon dioxide concentration ranged from 0.3 to 0.7 percent. The average oxygen concentration for the entire experiment was 15.7 percent. The nitrogen content was not determined. The growth under these conditions compared favorably with that under jars arranged as checks with similar concentrations of carbon dioxide, 0.4 to 0.8 percent, but with very slight dilutions of oxygen, 20.1 percent. This is shown in figure 3, *A*.

EXPERIMENT 2-B.—This experiment was essentially a duplicate of experiment 2-A. The concentration of carbon dioxide ranged from 0.2 to 1.0 percent, while the oxygen content ranged from 10.4 to 19.6 percent, averaging 16.3 percent. The effect of this treatment on growth is shown in figure 3, *B*.

EXPERIMENT 2-C.—In this experiment both carbon dioxide and oxygen were run into the jar in order to obtain a high concentration

of carbon dioxide in the presence of an excess of oxygen. The oxygen concentration ranged from 25.8 to 26.4 percent, while the carbon dioxide concentration ranged from 7.6 to 9.2 percent. The effect of the treatment is illustrated in figure 3, *C*.

EXPERIMENT 2-D.—This experiment was performed in an attempt to duplicate experiment 2-C. Carbon dioxide concentration ranged from 16.0 to 22.6 percent and oxygen from 26.4 to 26.6 percent. The effect on growth and weight are shown in figure 3, *D*.

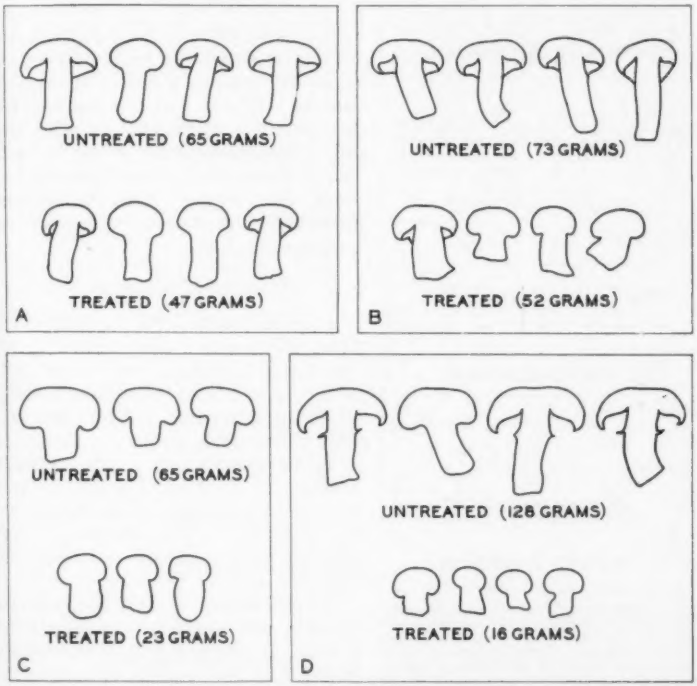

FIGURE 3.—Group *A* and group *B* show the effect of diluting the atmosphere with nitrogen. Groups *C* and *D* show the effect of high concentrations of carbon dioxide together with high concentrations of oxygen. Treated mushrooms in group *A* grew in an atmosphere with oxygen concentrations 7.8 to 20.0 percent and carbon dioxide 0.3 to 0.7 percent; group *B*, oxygen 10.4 to 19.6 percent, and carbon dioxide 0.2 to 1.0 percent; group *C*, oxygen 25.8 to 26.4 percent, carbon dioxide 7.6 to 9.2 percent; group *D*, oxygen 26.4 to 26.6 percent, carbon dioxide 16.0 to 22.6 percent. Untreated mushrooms grew in normal carbon dioxide and oxygen content of house.

EXPERIMENTAL SERIES NO. 3

The third series of experiments indicates that the stunting effect of an excess of carbon dioxide is due principally to its effect on the caps rather than on the mycelium in the compost.

EXPERIMENT 3-A.—Carbon dioxide was run slowly into the compost under a clump of button mushrooms growing in the open air on the bed. The carbon dioxide content reached 1.0 percent, but there was

no appreciable effect on the growth of the mushrooms, as shown in figure 4, *A*.

EXPERIMENT 3-B.—Carbon dioxide was run into the bed more rapidly than in experiment 3-A, and a higher carbon dioxide content resulted, 6.4 to 9.0 percent; yet there was no appreciable effect on the growth of the mushrooms. (See fig. 4, *B*.)

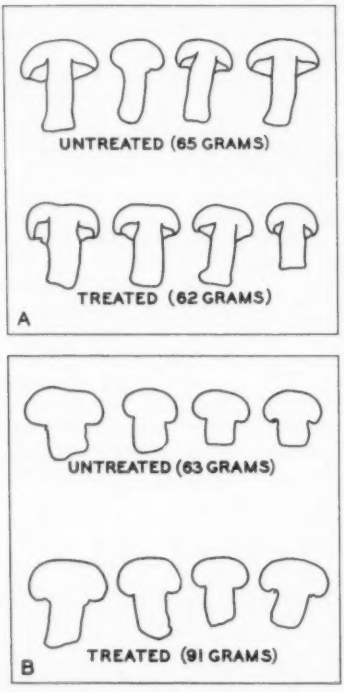

FIGURE 4.—Effect of high concentrations of carbon dioxide in the interstices of the compost under growing mushrooms. Under treated mushrooms in group *A*, carbon dioxide 1.0 percent; under treated group *B*, carbon dioxide 6.4 to 9.0 percent; untreated in both groups, carbon dioxide 0.3 to 0.4 percent. In all instances the sporophores grew in normal air.

EXPERIMENT 4

In an attempt to revive the mushrooms stunted by high concentrations of carbon dioxide in experiments 1-B and 1-C, several stunted mushrooms were left on the bed after the jars were removed. Growth was not resumed, and it was evident that the young mushrooms had been killed by the exposure to excess carbon dioxide.

EXPERIMENTAL SERIES NO. 5

It would seem from the fifth series that there is a slight tendency for the stems of mushrooms to elongate at a concentration of carbon dioxide of about 1 percent. At 1.8 percent this tendency is quite marked.

EXPERIMENT 5-A.—The concentration of carbon dioxide ranged from 0.4 to 0.8 percent and averaged 0.5 percent. The effect on the growth of mushrooms is shown in figure 5, *A*.

EXPERIMENT 5-B.—The carbon dioxide concentration ranged from 0.2 to 1.0 percent and averaged 0.6 percent. The results are shown in figure 5, *B*.

EXPERIMENT 5-C.—The carbon dioxide concentration ranged from 2.2 to 2.6 percent and averaged 2.3 percent. The results are shown in figure 5, *C*.

EXPERIMENT 5-D.—The carbon dioxide concentration was 0.9 percent. Results are shown in figure 5, *D*.

EXPERIMENT 5-E.—The carbon dioxide concentration was 0.6 percent. The results are shown in figure 5, *E*.

EXPERIMENT 5-F.—(Replication of 5-E.) The carbon dioxide concentration was 0.6 percent; a comparison of the treated and untreated mushrooms is shown in figure 5, *F*.

EXPERIMENT 5-G.—This experiment was similar to the others in this series, except that it was made on a floor bed in a cave at St. Paul,

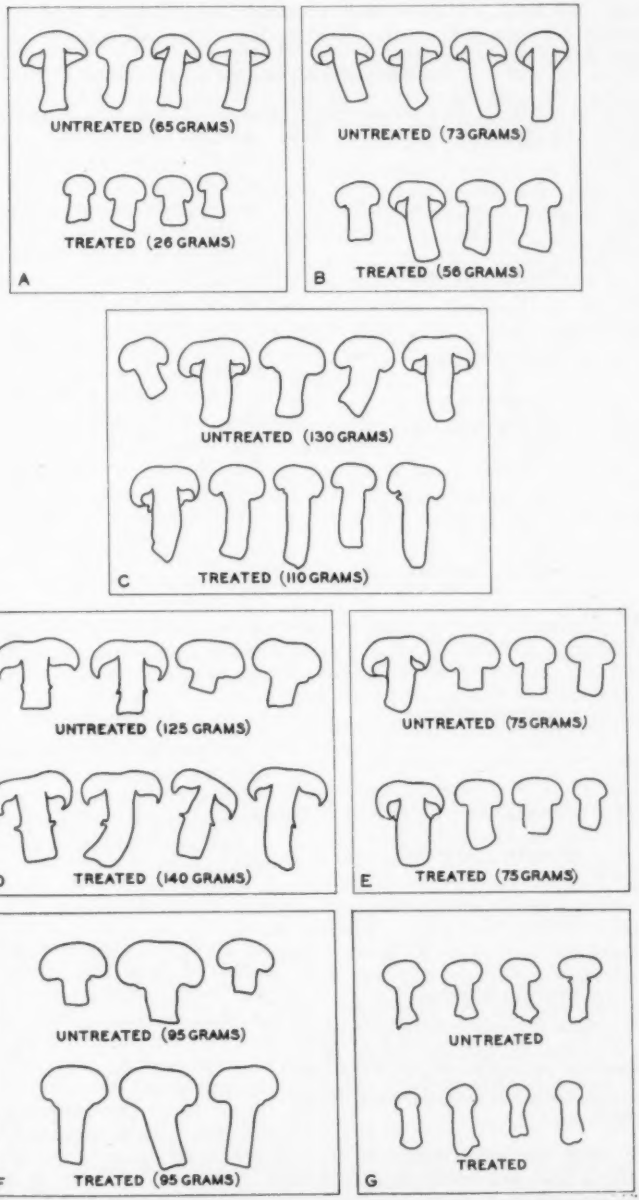

FIGURE 5.—Effect on the growth of mushrooms of small increases in concentration of carbon dioxide in the air. Treated sporophores in group *A* were subject to carbon dioxide average 0.5 percent; group *B*, carbon dioxide 0.6 percent; group *C*, carbon dioxide 2.3 percent; group *D*, carbon dioxide 0.9 percent; group *E*, carbon dioxide 0.6 percent; group *F*, carbon dioxide 0.6 percent; group *G*, carbon dioxide 1.8 percent untreated in all cases exposed to less than 0.1 percent carbon dioxide.

Minn., in 1931. The carbon dioxide accumulation under the jar reached 1.8 percent on the second day. The results are shown in figure 5, *G*.

EXPERIMENTAL SERIES NO. 6

In the sixth series a large excess of oxygen was run into jars containing clumps of mushrooms. There seemed to be a slight tendency for the mushrooms to be firmer and heavier in atmospheres containing a large excess of oxygen.

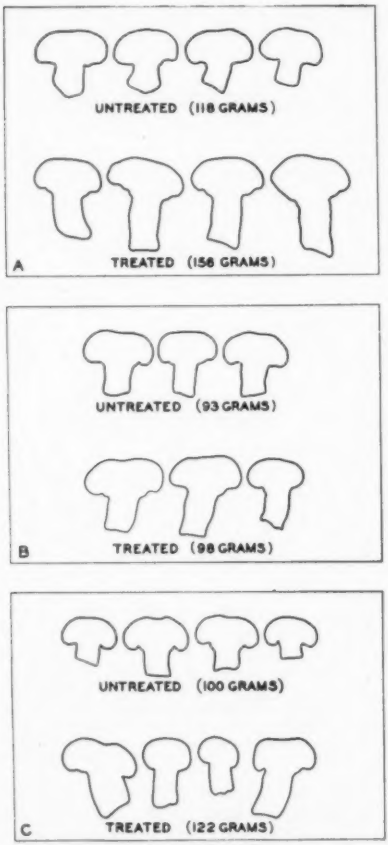

FIGURE 6.—Effect of an excess of oxygen on the growth of sporophores. In group *A* the treated mushrooms grew in an atmosphere containing approximately 37 percent oxygen and about 0.8 percent carbon dioxide; group *B*, oxygen 58 to 61 percent, carbon dioxide 0.4 percent; group *C*, oxygen 28 percent, carbon dioxide 0.6 percent.

EXPERIMENT 6-A.—The oxygen content under the jar ranged from 36.7 to 38.4 percent; the carbon dioxide ranged from 0.7 to 0.8 percent. A comparison of treated and untreated mushrooms is shown in figure 6, *A*.

EXPERIMENT 6-B.—The oxygen content under the jar varied from 58 to 61 percent; the carbon dioxide content was 0.4 percent. A comparison of the treated with the check mushrooms is shown in figure 6, *B*.

EXPERIMENT 6-C.—The oxygen content was 28 percent and the carbon dioxide 0.6 per cent. A comparison of treated with check mushrooms is shown in figure 6, *C*.

EXPERIMENT 7

An excess of oxygen was run into the compost under several clumps of mushrooms growing in the open air. There was no apparent stimulation from this treatment.

EXPERIMENT 8

Clumps of mushrooms were removed from the beds, and jars were inverted over these as well as over places in the bed containing tiny rudiments of mushrooms. After 5 days the carbon dioxide content under these jars averaged 0.2 percent. This is considerably lower than the concentration in series 5, indicating, as would be expected, that the sporophores liberate carbon dioxide freely during active growth.

OBSERVATIONS IN CAVES AND COMMERCIAL HOUSES

In the summer of 1931, samples of air were analyzed from different parts of three sandstone caves devoted to mushrooms culture at St. Paul, Minn. In one cave the beds were spawned but not yet in bearing. In this cave the carbon-dioxide content averaged only about 0.2 percent, in spite of the fact that the cave had been closed up tightly for several days. In the second cave there were actively growing mushrooms, but the door was left open at night for ventilation. The air in this cave had approximately the same carbon dioxide content, 0.2 percent. The third cave was dug somewhat closer to the outside wall and could not be kept cool during hot weather unless the doors were tightly closed. There were growing mushrooms in this cave, and after the door had been closed for a few days the carbon dioxide would reach a concentration of 1 percent. The mushrooms in this cave had a slight tendency to grow long stems with small caps as compared with those in the second cave.

In May 1932, air was analyzed from the mushroom houses of three commercial growers in Chester County, Pa. In all cases mushrooms were growing on the beds. After the doors had been closed for several hours the carbon dioxide content of the air at the ground level varied from 0.2 to 0.5 percent, and at the top of the house from less than 0.2 to 0.4 percent. Opening the doors overnight reduced the concentration to less than 0.1 percent.

DISCUSSION AND CONCLUSIONS

The foregoing experiments should perhaps be considered as field rather than as laboratory experiments. With a plant as variable as the cultivated mushroom, and with the comparatively crude methods used for maintaining and determining the composition of the atmosphere, it was not possible to establish precisely the limits of carbon dioxide and oxygen concentration tolerated by the mushroom. In spite of these limitations, however, the results of most of the experiments were clear-cut and justify definite conclusions.

When compared to the germinating spores and growing mycelium studied by Lopriore,[2] Brown,[3] Fellows,[4] and Neal and Wester,[5] the growing sporophores of cultivated mushrooms appear to be exceptionally sensitive to an excess of carbon dioxide in the air. Under the conditions of the experiments, concentrations of more than 5 percent carbon dioxide in the air arrested the growth of the sporophores and in some cases apparently injured them beyond recovery. At lower concentrations (2 percent, and perhaps even 1 percent) there was a tendency for the pileus to be retarded more than the stipe so that the mushrooms were out of proportion, having long stems and small caps.

It is of considerable interest that growing mushrooms are more sensitive to high concentrations of carbon dioxide surrounding the

[2] LOPRIORE, G. UEBER DIE EINWIRKUNG DER KOHLENSÄURE AUF DAS PROTOPLASMA DER LEBENDEN PFLANZENZELLE. Jahrb. Wiss. Bot. 28: [531]-636, illus. 1895.

[3] BROWN, W. ON THE GERMINATION AND GROWTH OF FUNGI AT VARIOUS TEMPERATURES AND IN VARIOUS CONCENTRATIONS OF OXYGEN AND OF CARBON DIOXIDE. Ann. Bot. [London] 36: [257]-283, illus. 1922.

[4] FELLOWS, H. THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF OPHIOBOLUS GRAMINIS IN PURE CULTURE. Jour. Agr. Research 37: 349-355, illus. 1928.

[5] NEAL, D. C., and WESTER, R. E. EFFECTS OF ANAEROBIC CONDITIONS ON THE GROWTH OF THE COTTON-ROOT-ROT FUNGUS PHYMATOTRICHUM OMNIVORUM. Phytopathology 22: 917-920, illus. 1932.

sporophores above ground than to similar concentrations in the interstices of the compost occupied by the mycelium. A possible explanation for this is that the comparatively loosely woven structure of the sporophore allows a direct diffusion of gases into the air from most of the hyphae, whereas the mycelium in the compost is buried deeply in the organic material through which it is growing and is therefore only partly in contact with the atmosphere of the compost. It may also be of some significance that when the hyphae of the sporophores are growing and respiring actively the mycelium in the compost has already finished most of its growth and is chiefly active in absorbing and translocating nutrients. In any case there is no justification for assuming that the evidence presented applies to young actively growing mycelium, or to sporophores in storage.

Analyses of gas in commercial houses and caves indicate that in practice harmful concentrations of carbon dioxide occur when the caves or houses have been closed up tightly for 24 hours or more. Theoretically the excess of carbon dioxide may be removed by air currents, by diffusion, or by absorption. Harmful concentrations of carbon dioxide can be avoided during the winter by opening the doors or ventilators for a few hours each day. In the case of summer crops grown in water-refrigerated houses where the air is recirculated to minimize the introduction of warm outside air it would seem advisable to make the water in the cooling sprays alkaline in order to absorb the accumulating carbon dioxide, and also to open the ventilators at the top of the house to allow for the escape of carbon dioxide by diffusion.

The evidence that a moderate dilution of oxygen is not detrimental to the growth of *Agaricus* is in agreement with the work of others, notably that of Porodko [6] and Brown,[7] which indicates that aerobic microorganisms usually are more tolerant of moderate reductions in oxygen pressure than of excesses of carbon dioxide.

SUMMARY

By running carbon dioxide under bell jars inverted over growing mushrooms (*Agaricus campestris* L.) it was found that an accumulation of 5 percent or more of carbon dioxide in the air caused abnormal growth, stunting, and even death of the mushrooms. Further experiments indicate that this effect was due to the presence of carbon dioxide and not to the dilution of oxygen. It was also demonstrated that the harmful effect was due principally to the carbon dioxide surrounding the sporophores rather than to that in the interstices of the compost in the bed. Approximately 1 percent of carbon dioxide was the lowest concentration that was noticeably injurious. This concentration was not encountered in commercial mushroom houses or caves except where they had been closed up tightly for 24 hours or more. An abnormally high concentration of oxygen seemed to produce more compact and heavier mushrooms. It is evident that mushroom houses should be ventilated as much as possible without interfering with temperature and humidity control or causing excessive evaporation from the beds by cross drafts.

[6] Porodko, T. Studien über den einfluss der sauerstoffspannung auf pflanzliche mikroorganismen. Jahrb. Wiss. Bot. 41: 1–64. 1904.

[7] Brown, W. (See footnote 3.)

EFFECT OF CERTAIN ENVIRONMENTAL FACTORS ON THE GERMINATION OF THE SPORANGIA OF PHYSODERMA ZEAE-MAYDIS[1]

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INTRODUCTION

In his studies of infection of corn plants by *Physoderma zeae-maydis* Shaw, Eddins (*2*)[3] noted that there was a wide variation in the incubation period following artificial inoculations, and concluded that these differences were probably due to the variation in time required for the sporangia to become exposed to conditions favorable for germination. He obtained higher percentages of infection in the field than in the greenhouse after artificial inoculation which indicated that certain environmental factors were more favorable for infection in the field.

According to Tisdale (*6*), the more essential factors influencing sporangial germination and zoospore formation of this organism are moisture, temperature, and fresh air. Additional factors that might possibly affect the development of a parasite are acidity of the medium, light, age of reproductive bodies, and gas content of the surrounding atmosphere. Several series of experiments were conducted in the laboratory and greenhouse to determine the effect of these factors on the germination of the sporangia in water cultures.

MATERIALS AND METHODS

Three hundred to five hundred sporangia collected from infected plants of corn (*Zea mays* L.) grown in 1932 were placed in each of several Syracuse dishes containing distilled water. These dishes in turn were placed in open chambers in the greenhouse, or on the laboratory table in moist chambers, in sealed desiccators, and in Novy improved anaerobic culture jars. The results given for each experiment represent an average of two trials run in duplicate. Unless otherwise stated, each experiment was conducted at a temperature between 27° and 30° C., which, according to Tisdale (*6*), comes within the range favorable for germination.

In a preliminary experiment the sporangia germinated equally well in tap water, distilled water, and corn-leaf extract. Therefore, in order to reduce to a minimum any variation in the acidity of the medium itself, distilled water was used in these experiments. Since it was also observed that the sporangia began germinating after 36 hours and showed the maximum percentage of germination at the end of 72 hours, the final counts were made in the remaining experiments

[1] Received for publication May 16, 1933; issued October 1933.
[2] Thanks are due Dr. A. H. Eddins for suggesting the investigation of this problem.
[3] Reference is made by number (italic) to Literature Cited, p. 473.

after 72 hours. The percentage germination was obtained by counting a total of 100 apparently normal sporangia in each dish. The sporangia were considered to have germinated when the lid or cap of the resting spores opened in doorlike fashion or was carried at the apex of the protruding endosporangium, as described by Tisdale (*6*).

FACTORS INFLUENCING THE GERMINATION OF THE SPORANGIA

FRESH AIR

In this test the Syracuse dishes containing the sporangia were placed in open chambers, moist chambers, and sealed desiccators on a laboratory table. The moist chambers were not sealed, which permitted the air to pass in and out of the chambers in places on the rim where the tops did not fit down tightly. The desiccators were sealed tightly with Lubriseal to prevent the exchange of the air between the outside and inside of the containers. In the open chambers and moist chambers, 87 percent germination was obtained; in the sealed desiccators 86 percent germination was obtained. This indicates that it is not necessary for the sporangia to be exposed to a constant supply of fresh air.

LIGHT

Germination tests were made with sporangia of *Physoderma zeae-maydis* by placing them in Syracuse dishes exposed to different light conditions. Part of the dishes were left open in the laboratory and in the greenhouse, and others were enclosed in moist chambers. The temperatures at which the containers were kept in different intensities of light were not the same in every case, but all of them came within the range favorable for germination.

The results of two trials (table 1) show that the intensity of light greatly affects the percentage of germination. Of the sporangia which received light from a north window in the laboratory 87 percent germinated, but of those in a dark chamber without light none germinated. Sporangia germinated in the presence of light from an electric lamp. This was demonstrated by placing them in a dark refrigerator adjusted to 10° C. and equipped with a 50-watt Westinghouse daylight bulb. By means of a small fan the cold air was drawn into the chamber containing the sporangia and kept circulating over the electric lamp. Thus, a constant temperature of 33° was maintained in the chamber, and 75 percent germination was obtained. Sporangia placed in open and in closed moist chambers on top of a greenhouse did not germinate. The sporangia in the open chambers were exposed to direct sunlight, while those in the closed chambers received sunlight through the glass cover of the chambers. No germination was obtained when the sporangia were placed in open chambers in the greenhouse 1 foot from the top and on a bench 8 feet from the top. In this case the sporangia were exposed to sunlight entering through the glass top of the greenhouse. Recording thermographs installed in the immediate vicinity of the containers showed that the temperature was favorable for germination in each case. The mean temperatures are shown in table 1. With all other conditions apparently favorable for germination, the foregoing results seem to indicate that light plays an important part in the germination of the sporangia.

TABLE 1.—*The relation of light to the germination of the sporangia of Physoderma zeae-maydis*

Place of exposure	Temperature	Percentage germination
	°C.	
Moist chamber on laboratory table	27	87
Darkened corner of laboratory	27	23
Complete darkness in chamber	30	0
Artificial light in dark chamber	33	75
Sunlight in closed moist chamber	32	0
Direct sunlight in open moist chamber	32	0
Greenhouse, 1 foot from top	30	0
Greenhouse, 8 feet from top	28	0

HYDROGEN-ION CONCENTRATION OF THE MEDIUM

The sporangia of *Physoderma zeae-maydis* were germinated in Syracuse dishes containing distilled water adjusted to different hydrogen-ion concentrations ranging from pH 2.1 to 8.1. The adjustments were made by adding the required number of drops of hydrochloric acid and sodium hydroxide of different normalities, the determinations being made with a Youden hydrogen-ion concentration apparatus. The sporangia germinated 88 percent at pH 7.4, which was the optimum reaction for germination. Germination was low at pH 3.1 and was inhibited at pH 2.5.

AGE OF THE SPORANGIA

Tisdale (*6*) has shown that the sporangia of *Physoderma zeae-maydis* remain viable over winter in the South. Eddins (*2*), in his studies on the longevity of the sporangia of this organism, has shown that sporangia stored for 3 years under different conditions were capable of causing from 41 to 85 percent infection when sprayed on corn plants.

In the present experiment, the sporangia were germinated in distilled water by the method described earlier in this paper. Some of the sporangia used were from the same bottles stored in the laboratory from which Eddins (*2*) had obtained the sporangia he used to inoculate corn plants. The sporangia stored in laboratory bottles for 1, 2, and 3 years germinated 70, 46, and 0 percent, respectively, as shown in table 2. The 3-year-old sporangia showed no germination in the laboratory but sporangia of the same age produced 41 percent infection when sprayed on corn plants in the field (*2*). This difference between the germination and the percentage of infection obtained may have been due to the stimulating effect of the presence of plant tissues on the spores, as noted by Durrell (*1*) for the spores of *Basisporium gallarum*, and Platz et al. (*5*) for the spores of *Ustilago zeae*.

TABLE 2.—*The effect of age of the sporangia of Physoderma zeae-maydis on their germination in distilled-water cultures*

Year sporangia were collected	Age of sporangia	Percentage of germination	Year sporangia were collected	Age of sporangia	Percentage of germination
1932	6 months	87	1929	3 years	0
1931	1 year	70	1928	4 years	0
1930	2 years	46			

GAS CONTENT OF THE SURROUNDING ATMOSPHERE

Oxygen Content

Platz (*4*) found that the percentage of germination of the spores of *Ustilago zeae* was affected by various percentages of oxygen and that the spores would not germinate in the absence of oxygen.

In testing the effect of various concentrations of oxygen in the surrounding atmosphere on the percentage of germination of *Physoderma zeae-maydis*, Syracuse dishes containing the sporangia were placed in Novy jars of a known volume, closed with ground-glass tops, and sealed with Lubriseal. The air was exhausted from the jars by means of a vacuum pump and the oxygen admitted into the jars from a gas cylinder was measured with gas burettes. After the desired percentage of oxygen had been admitted into each jar, air was admitted to make up the total volume. Thus, the gas in the jars was at normal atmospheric pressure. The pH reaction was determined at the beginning and at the end of the experiments. An attempt was made to germinate the sporangia of *Physoderma zeae-maydis* in the absence of oxygen by removing it from the Novy jars by the pyrogallic acid method (*7*).

The results (table 3) show that the percentage of germination of the sporangia is affected by the percentage of oxygen contained in the atmosphere. The highest percentage of germination was obtained in an atmosphere containing 20 percent of oxygen. It is interesting to note that this was the same percentage of germination as that obtained in the checks in normal atmosphere, which contains approximately 20 percent of oxygen by volume. The sporangia did not germinate in the absence of oxygen, whereas they germinated 80 percent in a normal atmosphere.

TABLE 3.—*The effect of oxygen on the germination of the sporangia of Physoderma zeae-maydis, and its effect on the pH of the medium, which was 6.9 at the beginning of the experiment*

Percentage of oxygen	Percentage of germination of sporangia	pH reaction of medium at end of experiment	Percentage of oxygen	Percentage of germination of sporangia	pH reaction of medium at end of experiment
Check	80	6.9	20	80	7.6
0	0	6.1	25	76	7.8
5	72	7.0	30	64	8.0
10	73	7.2	35	58	8.2
15	75	7.4			

The atmosphere containing higher percentages of oxygen produced greater alkalinity of the medium. In the oxygen-free atmosphere, the hydrogen-ion concentration of the medium was changed from pH 6.9 to 6.1. An atmosphere containing 20 percent of oxygen produced in distilled water a hydrogen-ion cencentration of 7.6, at which the maximum germination was obtained. This germination is comparable to that obtained at 7.4 produced by a known normality of sodium hydroxide in normal atmosphere.

Carbon Dioxide Content

Sporangia of *Physoderma zeae-maydis* were exposed to atmospheres containing various percentages of carbon dioxide. This was done in the same manner as in the preceding experiment with oxygen. The results of the two trials (table 4) show that the percentage of germination of the sporangia is affected by the percentage of carbon dioxide. Atmospheres containing 10 percent of carbon dioxide produced the highest percentage of germination, which is approximately the same as that obtained in a normal atmosphere and in that containing 20 percent of oxygen. No germination was obtained in the presence of a barium hydroxide solution, which probably absorbed all of the carbon dioxide in the containers. This same experiment was performed by Durrell (*1*), who found that spores of *Basisporium gallarum* also failed to germinate in the absence of carbon dioxide.

Table 4.—*The relation of carbon dioxide to the germination of the sporangia of Physoderma zeae-maydis, and its effect on the pH of the medium, which was 6.6 at the beginning of the experiment*

Percentage of carbon dioxide	Percentage of germination of sporangia	pH concentration of medium at end of experiment	Percentage of carbon dioxide	Percentage of germination of sporangia	pH concentration of medium at end of experiment
Check	80	6.6	20	69	5.6
0	0	6.9	25	32	5.3
5	72	6.3	30	7	5.1
10	79	6.1	35	0	4.8
15	76	5.9			

The pH concentration of the medium was changed by different concentrations of carbon dioxide. In atmospheres containing 10 percent of carbon dioxide, the pH of the medium was changed from 6.6 to 6.1. The percentage of germination produced at this concentration is very near that produced at pH 5.8, which was obtained by a known normality of hydrochloric acid in normal atmosphere. Unlike oxygen the higher percentages of carbon dioxide produced greater acidity of the medium. This is in accordance with results obtained by Kendall (*3*).

High and Low Temperatures

The thick-walled sporangia of *Physoderma zeae-maydis* are apparently not injured to any extent by the high summer temperatures and the cold, sometimes freezing, temperatures occurring in Florida. Eddins (*2*) found that sporangia stored in soil and leaf sheaths and exposed to the weather for 3 years remained viable and were capable of infecting over 50 percent of the corn plants inoculated. Tisdale (*6*) reported that some sporangia germinated after being allowed to freeze in a cake of ice for several days.

In an experiment conducted to determine the effect of high temperature upon the viability of sporangia, the sporangia were placed in Syracuse dishes without any water and placed in electrically controlled dark ovens held at constant high temperatures, as noted in table 5. Other sporangia were placed in Syracuse dishes containing water, which were placed in a refrigerator at 0° C. and allowed to freeze.

At 2-, 15-, and 30-day intervals, a number of the dishes were removed from the refrigerator and dark ovens. Water was added to them, and they were placed on a laboratory table at room temperature (30° C.) to germinate.

It was found that the sporangia are tolerant of constant high and low temperatures (table 5); they even germinated after being exposed to temperatures of 70° C. and 0° C. for 30 days.

TABLE 5.—*The effect of high temperature and freezing on the germination of the sporangia of Physoderma zeae-maydis*

Days exposed	Percentage germination after exposure to—					
	0° C.	40° C.	50° C.	60° C.	70° C.	80° C.
2	86	86	86	85	84	64
15	45	51	46	45	42	0
30	7	40	37	35	28	0

SUMMARY

The effect of certain environmental factors on the germination of the sporangia of *Physoderma zeae-maydis* Shaw was studied, from 300 to 500 sporangia collected from infected corn plants being used. The sporangia were placed in Syracuse dishes containing distilled water and were subjected to various controlled environmental conditions. A preliminary experiment had shown that the sporangia germinated equally well in tap water, distilled water, and corn-leaf extract.

Sporangia germinated almost equally well when exposed in sealed and nonsealed containers, indicating that a continuous exchange of fresh air is not essential for germination. They were found to germinate best in a moist chamber on a laboratory table when they received light from a north window. They also germinated well in a dark chamber under artificial light but did not germinate at all in total darkness. Direct sunlight appeared to be lethal.

In distilled water adjusted to pH 2.5 the sporangia did not germinate. The optimum reaction was pH 7.4, at which 88 percent germination was obtained.

When stored in laboratory bottles for 1, 2, and 3 years sporangia germinated to the extent of 70, 46, and 0 percent, respectively. However, previous investigations had shown that sporangia stored in laboratory bottles for 3 years produced 41 percent infection when sprayed on corn plants in the field.

Sporangia did not germinate in the absence of oxygen but germinated to the extent of 80 percent in the presence of 20 percent oxygen, which is the same percentage germination as that recorded for normal atmosphere. In the atmosphere containing 20 percent oxygen a pH value of 7.6 was produced in distilled water that had had a pH value of 6.9 at the beginning of the experiment.

Germination was best in atmospheres containing 10 percent of carbon dioxide, being 79 percent—only 1 percent less than that of the control. The pH value of the medium was changed from 6.6 to 6.1 in this case.

The sporangia were tolerant of high temperatures. They even germinated after being exposed to temperatures of 0° and 70° C. for 30 days, though there was no germination after 15 days, or after 30 days' exposure to a temperature of 80°.

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A TYPE OF LABORATORY SILO AND ITS USE WITH CROTALARIA[1]

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INTRODUCTION

In regions where the usual forage crops are poorly adapted and climatic conditions render haymaking uncertain, the use of new crops and methods for their preservation must be further investigated. Various species of the genus *Crotalaria* have been found to be well adapted culturally to the lighter sands of the Coastal Plains region (*16*)[3] and are comparable in composition to the other legumes used in livestock feeding. The success attained at the Florida station in ensiling soybeans (*5*), the seasonal distribution of rainfall (*17*) which makes natural curing of hay an unsatisfactory practice, and the independence of silage-making of weather conditions, suggested the desirability of determining the ensilability of the crotalarias.[4] Preservation of forage as silage has the further advantages of economy of labor and storage space (*9*), reasonable efficiency of conservation of nutrients and succulence, and an increase in palatability brought about by the ensiling process.

The experiments were planned to test the merits of a type of silo which was developed for this work. Four of the silos were constructed so that several species of *Crotalaria* might be studied concurrently with a minimum expenditure of materials and labor, preparatory to more exact studies of digestibility and feeding value.

WORK OF OTHER INVESTIGATORS

The first report in American literature of the ensiling of forage in experimental quantities with determinations of nutrient losses was made by Jordan (*14*) at the Pennsylvania station in 1884. Watertight tubs 6 feet in height and 3 feet in diameter were filled with chopped sorghum and weighted to secure packing.

Cooke (*7*) reported the use of tubs 3 feet in height and 2 feet in diameter, holding about 350 pounds of maize, at the Vermont station in 1889. A layer of roofing paper and a wooden disk weighted with stones to equal 50 pounds per square foot prevented all surface spoilage. A loss of 4.08 percent in total weight and 14.67 percent in dry matter was observed. Hills (*13*) later made a satisfactory silage from sugar beets and straw in these silos.

[1] Received for publication May 31, 1933; issued October 1933.
[2] Grateful acknowledgment is made to Will T. Dunn for analytical services and to P. T. Dix Arnold for conducting the palatability tests.
[3] Reference is made by number (italic) to Literature Cited, p. 623.
[4] The forage ensiled was provided under the terms of a cooperative agreement between the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Agronomy and Animal Husbandry Departments of the Florida Agricultural Experiment Station. G. E. Ritchey was in charge locally for the Division of Forage Crops and Diseases.

The early work at the Wisconsin station with silage (*12*, *24*) was done with rectangular silos, 7 by 8 feet and 14 feet deep, with a capacity of approximately 10 tons of forage. Clover was ensiled successfully with losses of 15.4 percent of dry matter and 12.7 percent of crude protein. Lawes and Gilbert (*15*) had ensiled clover as well as meadow grass in the pit silos at Rothamsted with smaller losses of nutrients.

A series of studies of the adaptation of many of the forage crops for making silage was instituted at the Missouri station by Eckles (*8*, *10*) in 1915. The final results were tabulated by Ragsdale and Turner (*21*). Cypress-stave silos 6 feet tall and 3 feet in diameter were filled with corn, oats and cowpeas, cowpeas, soybeans, alfalfa, Sudan grass, wheat, oats, rye, and sunflowers. All but rye made good silage although it was recommended that the legumes be ensiled only when they had wilted sufficiently to give a 30 to 35 percent dry-matter content. A wire screen was placed in the silo when it was within 18 inches of being full. The silos were sealed with a felt-lined wooden disk and weighted with 1,000 to 1,500 pounds of rock. The weight and composition of the forage placed under the screen as compared with the same data for the silage, were used to measure the unavoidable losses in the ensiling of the crops.

Reed and Fitch (*22*) studied the ensilage of alfalfa in upright silos 7 feet in diameter and 16 feet high, made from 1-inch flooring. Alfalfa alone made poor silage. The addition of carbohydrates in the form of meal, molasses, or sorghum increased the acid production and improved the quality. Swanson and Tague (*25*, *26*), using quart milk bottles as containers, made parallel studies with alfalfa and sweetclover. Carbohydrate additions were beneficial and sweetclover was more desirable than alfalfa for making silage. True, Woll, and Dolcini (*27*) encountered no difficulty in making good silage from alfalfa alone in a commercial silo. This could be taken to indicate that conditions in the experimental silos only approximated those in larger silos.

Westover and Garver (*28*) ensiled 150 to 200 pounds of alfalfa, sweetclover, corn, sorghum, Sudan grass, wild sunflowers, Russian thistles, and corn or sorghum mixed in equal parts with alfalfa, in oil barrels, reheading the barrels after they were filled. Neither the sunflower nor thistle silage was palatable.

Blish (*6*) utilized galvanized-iron cylinders 23 to 27 inches in diameter and 7.5 feet high in his studies of factors affecting the quality of sunflower silage at the Montana station. A good-quality silage was produced except with sunflowers low in sugars. Peterson, Hastings, and Fred (*20*) likewise used iron cylinders 4 feet in diameter and 8 feet high, when investigating the changes that take place in the making of silage.

Woodman and Amos (*1*, *2*, *29*, *30*, *32*) used wood-stave silos 6 feet high and 3 feet in diameter in a series of investigations with English forage crops. The forages included oats and vetch, maize, sunflowers, sugar-beet tops, and a mixture of oats, vetch, and beans. Metal extensions were placed on the silos during filling. The silage was covered with a layer of earth to pack it and to decrease spoilage. Sample bags buried in each silo at two different levels were used for the determination of changes in composition and losses of nutrients.

Losses with the mixed silages in the experimental silos were comparable to those in commercial silos (*1, 29, 33*) and were smaller than those in either clamp (*3*) or stack (*31*) silage.

Even with the comparability of losses between the experimental silos and commercial silos as found by Woodman and Amos with mixed silages, and as found by Eckles (*8*) with maize, the complete comparability of the two types of silos may be questioned. Eckles found the legumes should have a dry matter content of 30 to 35 percent for successful ensilage in the experimental silos, while a dry-matter content of 25 percent with soybeans was found sufficient at the Florida station (*18*). However, all the evidence indicated the value of the laboratory silo as a tool with which to study the ensilability of forage crops.

EXPERIMENTAL METHODS AND MATERIALS

Four small pit silos were constructed in a hillside having good surface drainage. They were 43 inches in inside diameter without any taper, and between 74 and 81 inches in depth, with smooth concrete walls, and clay and limerock bottoms.

Each silo was filled with one of the crotalarias on September 22–23, 1932. The forage was cut in 1½-inch lengths in a silage cutter, weighed in tubs, and packed in the silo by tramping and tamping. When the top of the silo was reached, a piece of burlap was placed over the surface, additional cut material was packed over it, and finally the whole was covered with approximately 2 feet of earth. This earth layer was cone-shaped to carry off the rainfall. (See fig. 1.) The silos and manner of filling were designed to simulate as closely as possible the conditions found in large silos.

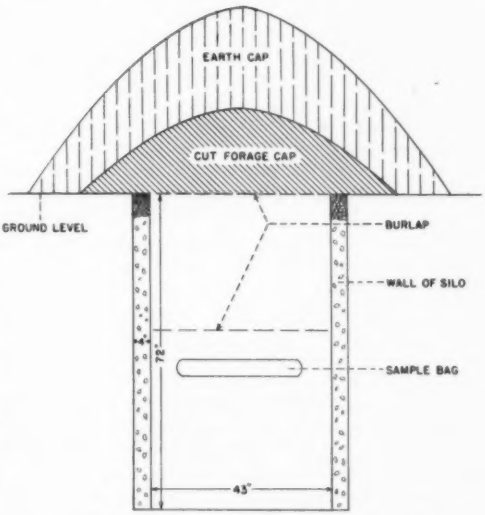

FIGURE 1.—Cross section of laboratory silo when filled.

A 10-kg sample of the forage in a muslin bag was buried about midway in depth in the silo as it was being filled. This was covered by a thin layer of crotalaria, over which a moist piece of burlap was placed as a marker. A 1-kg sample representative of the forage in the muslin bag was taken for analysis.

The silos were opened on December 12, 1932, 81 days after they were filled. The earth and silage were stripped off to the burlap

cover. Spoiled silage underneath the burlap was removed and weighed. Good silage was removed, weighed, and offered to cattle over a 16-day period to determine its palatability. The small bags were weighed as they were recovered, and sampled for analysis.

Analyses were made by the methods of the Association of Official Agricultural Chemists (*4*). Juice for the hydrogen-ion determinations was obtained by pressing samples of the fresh silage, and the determinations were made with a quinhydrone electrode. Losses of nutrients were calculated from the weight and composition of the forage placed in and the silage removed from the sample bags.

Four species of *Crotalaria* were used to test the feasibility of these laboratory silos and the ensilability of the forages, namely. *C. spectabilis* Roth, *C. striata* DC., *C. intermedia* Kotschy, and *C. incana* L. All were further advanced in growth than is usually considered desirable for hay, with flowers and many seed pods present. Many of the lower leaves of *C. spectabilis* and *C. incana* had fallen.

PRESENTATION OF DATA

The quantity of forage ensiled, the quantity of spoiled and sound silage recovered, and the density of the silage at each foot in the silo for each species, are presented in table 1. It will be observed that from 2,088 to 2,609 pounds of forage were cut, weighed, and packed in the silos, and from 47 to 241 pounds of spoiled silage were removed from beneath the top piece of burlap 81 days later when the silos were opened. From 1,854 to 2,329 pounds of sound silage were removed during the next 16 days. The forage over the burlap was not weighed but was estimated at 500 pounds. Over the *Crotalaria striata* silo much of this material was unspoiled, but over the others it did not keep.

TABLE 1.—*Crotalaria forage ensiled and recovered as silage in laboratory silos, together with weights per cubic foot of the settled silage*

[Forage ensiled Sept. 22–23, 1932; silos opened Dec. 12, 1932]

Species of *Crotalaria*	Forage ensiled	Silage		Depth of silo	Depth of silage	Silage density per cubic foot				
		Spoiled	Sound			First foot	Second foot	Third foot	Fourth foot	Fifth foot
	Pounds	*Pounds*	*Pounds*	*Inches*	*Inches*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
C. spectabilis	2,609	233	2,227	80.50	58.0	40.75	42.86	44.16	44.72	[a] 45.44
C. striata	2,420	47	2,322	76.00	54.5	36.55	40.69	43.15	43.92	44.16
C. intermedia	2,526	131	2,329	80.75	67.0	36.20	38.09	39.28	40.76	42.80
C. incana	2,088	241	1,854	74.00	53.0	38.71	40.52	41.17	41.53	.....

[a] Gravitational moisture evident.

The changes that took place in the forage were determined by comparing the weight and composition of the forage ensiled with the weight and composition of the silage recovered in the sample bags. The composition of the forage and silage, and the percentage recovery of the various constituents of the forage ensiled are presented in table 2.

TABLE 2.—*Composition of fresh Crotalaria forage and Crotalaria silage, and percentage recovery of the nutrients of the forage in laboratory silos*

Species of *Crotalaria*	Net weight of material	Moisture	Dry matter	Composition of the dry matter					pH values
				Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	
CROTALARIA FORAGE									
	Kilograms	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
C. spectabilis	10.000	79.14	20.86	6.89	10.00	41.70	40.03	1.38	.....
C. striata	10.000	74.08	25.92	3.89	17.03	39.14	37.90	2.04	.....
C. intermedia	10.000	75.00	25.00	5.24	12.04	41.00	40.08	1.64	.....
C. incana	10.000	75.68	24.32	5.81	11.40	46.40	35.12	1.27	.....
CROTALARIA SILAGE									
C. spectabilis	9.946	81.84	18.16	7.50	10.18	46.29	33.95	2.08	4.66
C. striata	9.432	76.58	23.42	4.43	14.20	44.39	33.74	3.24	5.50
C. intermedia	9.835	77.64	22.36	5.73	10.74	46.80	33.82	2.91	5.50
C. incana	9.312	76.46	23.54	6.63	9.94	47.92	33.08	2.43	5.04
(Corn silage)									3.47
PERCENTAGE RECOVERY OF THE CONSTITUENTS									
C. spectabilis	99.46	102.85	86.59	94.25	88.14	96.12	73.44	130.51	.....
C. striata	94.32	97.50	85.22	97.05	71.06	96.65	75.87	135.35	.....
C. intermedia	98.35	101.81	87.96	96.19	78.47	100.41	74.23	156.08	.....
C. incana	93.12	94.08	90.13	102.85	78.59	93.09	84.90	172.46	.....

The crotalaria forage ensiled in the laboratory silos was found to have undergone the typical changes encountered in large silos with other crops. The destruction of protein was not so great, but the losses of dry matter and nitrogen-free extract were greater than had been obtained previously by the writers (*18*) with soybeans. The increase of ether extract was more marked. There had been some leaching of soluble ash. The silage had a pungent acid odor, and hydrogen-ion measurements showed it to be acid, although less so than corn silage, which was found to have a pH value of 3.47.

Quantities of the different silages were offered to growing dairy heifers to determine the relative palatability. All four of the crotalaria silages were placed in compartments of the same feed bunk, and the order changed from day to day. Records taken were limited to notations on order of choice and approximate amount refused.

Crotalaria incana and *C. intermedia* silages were eaten immediately and with almost no waste. *C. spectabilis* was eaten after the first two had been taken, and without significant refusal.[5] The *C. striata* silage was refused almost completely by all of the animals (14 head) used in this study.

[5] Unpublished work indicates a toxic principle in the seeds of *C. spectabilis* when eaten by chickens. The forage also may be poisonous, but the limited quantities of the silage offered caused no visible symptoms of poisoning. Further toxicity studies are in progress.

DISCUSSION OF RESULTS

This investigation was planned to determine whether or not such laboratory silos as are described could be used in preliminary studies with forages, and also to ascertain whether or not any of the crotalarias might be desirable as silage crops. Such an investigation was necessary because the climatic conditions in Florida favor the preservation of forages as silage rather than as naturally cured hay.

The silos and the manner of filling them were designed to simulate the conditions in large silos with a minimum expenditure of labor and materials. The use of the pit type of silo, which could be capped easily with additional forage and earth, was an important feature. Leakage of air through the walls was minimized and the weight of the cap of forage and earth aided in packing the silage. How well this was accomplished is shown by the density of the silage, in table 1. The surface foot was of a density equivalent to corn silage at the eighth foot (*11*), or of soybean silage at the fifth foot (*5*). The loss by spoilage of the crotalaria forage actually placed in these silos was very small.

If the changes in composition and the recovery of nutrients in the laboratory silos were equivalent to those found with other crops in large silos, it could be concluded that a typical silage had been produced. If such a silage were produced, tests of its palatability would determine whether or not additional investigation of the feeding value of the silage was justified.

The demonstration in commercial practice that good silage may be made from the legumes alone, the indifferent success with alfalfa in experimental stave silos at the Kansas station (*22*), and the probable necessity of a dry-matter content of 30 to 35 percent in legumes for ensilage at the Missouri station (*8*) would indicate that commercial conditions had not been achieved in those types of experimental silos used in testing the ensilability of legumes. Chemical studies of the nutrient losses in the Kansas investigation were not presented, but the legumes of a higher dry-matter content at the Missouri station underwent normal changes and suffered the expected losses of nutrients.

In the laboratory silos filled with crotalaria the loss of dry matter was only slightly greater than that found with corn silage (*19*, *20*, *23*) and soybean silage (*18*) in commercial silos. The recovery of crude protein was good, and the increase in ether extract indicated that the fermentations resulting in ether-soluble acids had occurred, as found in normal ensilage. These results were secured with forage containing from 20.9 to 25.9 percent of dry matter from which silages containing 18.2 to 23.5 percent of dry matter were obtained. The silage had a pungent acid odor, and from both physical appearance and chemical composition, could be considered typical.

The palatability studies showed three of the four species to be much more desirable as silage than as either green forage or hay.[6] Not only were they eaten readily, but the amounts refused were insignificant. *Crotalaria striata* has not seemed to be adapted as a livestock feed, and careful toxicity studies of *C. spectabilis* are necessary. Final selection of a particular species for ensilage must depend on additional tests with other species, as well as on the consideration of

[6] Unpublished material from another phase of the cooperative investigation.

yields, freedom from insect pests and diseases, and similar matters of an agronomic nature. All of the species studied apparently underwent typical ensilage changes to a degree that would be expected in large silos. The possible use of *C. incana* and *C. intermedia* is indicated definitely. However, they should not be recommended without additional investigation.

The potential place of a slightly larger silo of the type used in this experiment, with a capacity sufficient to supply silage for digestibility studies is worthy of consideration.

SUMMARY AND CONCLUSIONS

Typical silage was produced in laboratory pit silos of 2,000 to 2,500 pounds capacity with crotalaria forage. Chemical changes and losses of nutrients were of the same order as usually are encountered in large silos with other crops. Conditions more nearly comparable to those in large silos were secured than appear to have been obtained heretofore by others working with small experimental silos of other kinds in the ensiling of legumes.

Crotalaria incana and *C. intermedia* forages made palatable silage which was eaten readily by cattle. *C. spectabilis* silage also was eaten readily, but its possible toxicity requires further investigation. *C. striata* does not seem to be adapted for use as a livestock feed.

Even though some species of the genus *Crotalaria* may be ensiled with only reasonable losses and the resulting product may be palatable to cattle, there yet remains the testing of other species in the manner herein described, the consideration of cultural characteristics of the various species, and an evaluation of crotalaria silage as a livestock feed, before any recommendations can be made concerning the use of crotalaria as silage.

The potential place of a larger laboratory silo of the type used in these experiments is suggested.

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THE VITAMIN C CONTENT OF COMMERCIALLY CANNED SAUERKRAUT PRODUCED UNDER KNOWN CONDITIONS [1]

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INTRODUCTION

In a previous paper [3] the antiscorbutic value of six brands of commercially canned sauerkraut was shown to vary widely. The vitamin C content of two of these brands compared very favorably with specially fermented fresh raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin,[4] while the poorest brand contained from one half to one third as much vitamin C as the best brands. Because of the lack of uniformity in the methods used in the manufacture of the sauerkraut tested, it was not possible to determine what factor or set of factors was responsible for the loss of antiscorbutic vitamin in the poorer brands. The cooperation of 4 of the 6 commercial packers who had contributed to the previous experiment was welcomed, therefore, in producing samples of canned sauerkraut under controlled conditions, as nearly uniform as feasible in all four factories. The packers who had produced the two highest scoring brands, A and B, the previous year, did not share in the present experiment.

Earlier observation [5] that bulk sauerkraut, as purchased, may be very poor in antiscorbutic potency was confirmed by the experiments of Lawrow and Jarussowa [6] who tested a market lot of sauerkraut which they kept in a wooden keg. The juice fed was prepared by first draining off and discarding the brine, and then squeezing the chopped sauerkraut in a cloth by hand. Thirty cubic centimeters of this juice fed daily gave complete protection against scurvy in guinea pigs, but 20 cubic centimeters gave only partial protection. The author estimated that the sauerkraut itself, without the brine, contained about 70 percent of the concentration in the juice tested.

[1] Received for publication Feb. 27, 1933; issued October 1933. This study was made possible through an industrial fellowship fund given to the University of Wisconsin by the National Kraut Packers' Association. The research carried out under the fund was organized and executed by members of the university staff in the university laboratories.

[2] Thanks are due to Dr. W. H. Peterson, Department of Agricultural Chemistry, and Dr. E. B. Fred, Department of Agricultural Bacteriology, for valuable assistance in planning the research; to W. I. Berg and other members of the research committee of the National Kraut Packers' Association for outlining the detailed procedures to be followed in the manufacture of the sauerkraut and securing the cooperation of the four sauerkraut packers; and to the packers themselves for their courtesy in supplying the data on the manufacturing procedures used. Thanks are also due Dorothy Adgate and Corrine Hogden for assistance in the preparation of the solutions, in calibration, and in chemical determinations. All solutions were checked by the senior author.

[3] CLOW, B., PARSONS, H. T., and STEVENSON, I. THE VITAMIN C CONTENT OF COMMERCIALLY CANNED SAUERKRAUT, TOGETHER WITH SOME OBSERVATIONS ON ITS VITAMIN A CONTENT. Jour. Agr. Research 41: 51-64, illus. 1930.

[4] ——— MARLATT, A. L., PETERSON, W. H., AND MARTIN, E. A. THE VITAMIN C CONTENT OF FRESH SAUERKRAUT AND SAUERKRAUT JUICE. Jour. Agr. Research 39: 963-971, illus. 1929.

[5] ELLIS, N. R., STEENBOCK, H., AND HART, E. B. SOME OBSERVATIONS ON THE STABILITY OF THE ANTISCORBUTIC VITAMINE AND ITS BEHAVIOR TO VARIOUS TREATMENTS. Jour. Biol. Chem. 46: 367-380, illus. 1921.

[6] LAWROW, B. A., and JARUSSOWA, N. SAUERKRAUT ALS C-VITAMINTRÄGER. Biochem. Ztschr. 229: 115-127, illus. 1930.

PLAN OF EXPERIMENT

As it was desirable that the details of the method employed in producing the canned sauerkraut should be in accordance with the best commercial practice, an outline of the processes necessary to be controlled in the experiment was drawn up by members of the Departments of Home Economics, Agricultural Chemistry, and Agricultural Bacteriology of the University of Wisconsin and submitted to a research committee of the National Kraut Packers' Association for their decision as to the exact procedure to be followed. Specific detailed instructions were then sent to each of the four factories. The points covered in the instructions are indicated by the details under item in table 1. Machine canning, which presumably would give the most nearly accurate control possible in commercial practice, was employed by three of the plants. The fourth plant, however, was particularly interested in hand packing, and inasmuch as large quantities of commercial sauerkraut are packed by hand, it was thought that the inclusion of this method would make the samples more nearly representative of commercial sauerkraut and furnish a valuable control. Except for this one variation in the method of canning, an attempt was made to secure the highest degree of uniformity possible throughout the four factories.

TABLE 1.—*Condensed reports of the four sauerkraut companies on methods employed in manufacturing and canning the brands used in present experiments*

CUTTING

Item	Month	Brands [a]			
		C_2	E_2	F_2	D_2
Variety of cabbage used		All Seasons	All Seasons	Believed to be mostly Glory of Enkhuizen.	All Seasons 70 percent, All Head, Glory of Enkhuizen.
Salinometer readings at time of filling tank.		15° F	November 15° F March, 19° F	15° F	November, 16.5° to 15° F. April, 15°.

TEMPERATURE

Room temperatures during fermentation	November December January February March	68°	72° to 48° F 61° to 38° F 38° to 26° F 53° to 30° F 62° to 54° F	55° to 70° F. (range for entire time).	56° to 44° F. 63° to 46° F. 58° to 36° F. 62° to 48° F. 59° to 50° F.
Temperature of the cabbage at time of cutting.		Estimated to be 45° to 50° F.	42° to 44° F	47° to 56° F	44° to 46° F.
Temperature of sauerkraut during fermentation.	November December January February March	55° F. (estimated)	56° to 48° F 48° to 44° F 43° to 30° F 40° to 30° F 49° to 44° F	70° to 61° F 70° to 66° F 65° to 55° F	56° to 44° F. 55° to 49° F. 54° to 42° F. 51° to 46° F. 53° to 50° F.
Temperature of cold sauerkraut at time of canning.		46° F	45° to 49° F	65° F	51° F.

[a] Listed in order of vitamin C concentration.

TABLE 1.—*Condensed reports of the four sauerkraut companies on methods employed in manufacturing and canning the brands used in present experiments*—Continued

ACIDITY

Item	Month	Brands					
		C_2	E_2	F_2	D_2		
					Top	Middle	Bottom
					Percent	*Percent*	*Percent*
Average acidity of fermenting sauerkraut for each month, expressed as lactic acid	November				0.72	0.68	0.74
	December				1.25	1.24	1.30
	January				1.31	1.32	1.32
	February				1.52	1.54	1.57
	March				1.66	1.67	1.69
Acidity of the bulk sauerkraut as it goes into the can.		2.16 percent	1.46 percent [c]	1.66 percent	[c] 1.72	[c] 1.72	[c] 1.76
		1.98 percent [b]	1.46 percent [b]				
Total acidity, expressed as percentage lactic acid, in the canned sauerkraut (average for 20 determinations for each brand).		1.47 percent	1.14 percent	1.40 percent	1.39 percent.		
Highest reading of acidity in cans [b]		1.49 percent	1.22 percent	1.43 percent	1.43 percent.		
Lowest reading in acidity in cans [b]		1.45 percent	1.10 percent	1.37 percent	1.36 percent.		
Reduction in acidity during canning		29 percent	22 percent	16 percent	20 percent.		
pH of canned sauerkraut [b]		5.2	5.2	5.2	5.2.		

CANNING

Length of fermentation		121 days	130 days	92 days	145 days.		
Date of opening tank and canning		March 6	March 17	February 19	April 2.		
Time in preheating tank		Estimated to be 6 minutes	Approximately 20	4 minutes	10 minutes.		
Temperature of sauerkraut as it leaves preheating tank and is put into cans.		180° F	132°–137° F	110° F	130° F.		
Method of packing		Machine	Machine	Hand	Machine.		
Additional brine or water used in canning		Mixture of about 3 barrels salt water to each 15 barrels of sauerkraut in preheating vat.	Mixture of 5 percent brine and 95 percent pure water added in automatic filler.	Hot salt brine (not sauerkraut brine) in preheating vat.	Approximately 3 ounces water added to each can in the filler.		
How the amount of brine added is determined.			To control acidity (tests at various intervals).	Added to keep up the heat			
Time in exhaust			5 minutes	5 minutes	7 minutes		

Time in exhaust			5 minutes	5 minutes	7 minutes.
Temperature in the can before sealing		Average 178° F.	146° F. bottom 140° F. center 186° F. outside	120° F. center	140° F. center. 184° F. outside.
Cooking after sealing		None	None	Cooked in rotary Anderson and Barngrover continuous rotary cooker for 10 minutes; center of can after cooking was 165°.	None.

SOLID AND LIQUID CONTENTS OF COMMERCIALLY CANNED SAUERKRAUT

Average weight of the drained solid content of sauerkraut in 16 or more cans of each brand.		681 grams	675 grams	669 grams	717 grams.
Average weight of liquid in 16 cans of each brand.		103 grams	131 grams	138 grams	89 grams.
Additional liquid required to completely fill cans of sauerkraut:					
A, range		28 to 55 cubic centimeters	28 to 46 cubic centimeters	25 to 56 cubic centimeters	37 to 60 cubic centimeters.
B, average of the six determinations made for each brand.		38 cubic centimeters	39 cubic centimeters	42 cubic centimeters	43 cubic centimeters.
Reduced pressure in cans of sauerkraut; average of three determinations for each brand.		8 pounds	12.1 pounds	11.6 pounds	12.8 pounds.

[b] Acidity tests made at University of Wisconsin. Average acidity of C_2 was 2.07 percent.
[c] Figures for last determination of acidity in fermentation tank.

EXPERIMENTAL PROCEDURE

The details of the care of the experimental animals and the feeding of the doses of sauerkraut were identical with those employed by Clow, Parsons, and Stevenson.[7]

Standard solutions and calibrated glassware and thermometers were furnished to the four companies by the Department of Home Economics of the University of Wisconsin.

The hydrogen-ion content of the canned sauerkraut was determined by means of a Leeds and Northrup potentiometer with a quinhydrone electrode.

Total acidity was determined by titrating with N/10 sodium hydroxide 10 cc of sauerkraut juice after the addition of 15 cc of water and 1 cc of a 1 percent solution of phenolphthalein and after a few seconds of boiling.

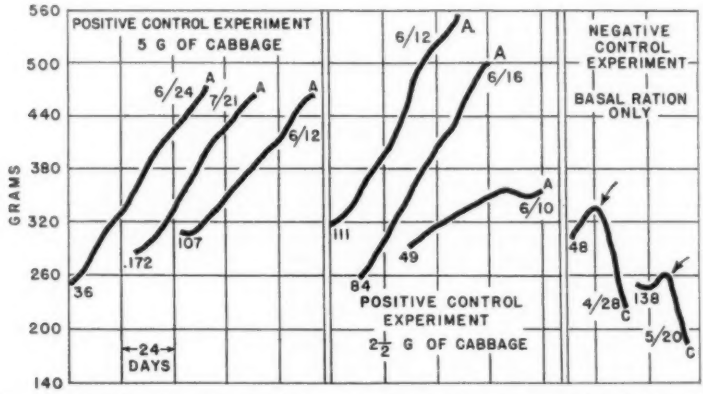

FIGURE 1.—Control experiments on vitamin C. In the positive control experiment, the animals received 5- or 2½-g doses of raw cabbage daily. In the negative control experiment, the animals were fed the basal ration alone; arrows above the weight curves indicate the point at which pronounced scurvy became evident. The month and day of the month on which autopsy was performed are indicated near the end of the weight curve of each animal. Autopsy symbols are as follows: A, no sign of scurvy; A?, possibility of scurvy; A−, slight but definite signs of scurvy; B+, moderate scurvy; B, B−, C+, and C, increasing degrees of scurvy.

Determinations of the solid contents of the cans of sauerkraut were made as follows: Each can of sauerkraut, upon being opened, was rapidly spread on a 9 by 4 inch piece of wire gauze of ¼-inch mesh, suspended in a metal pan into which the juice drained. The total weight of sauerkraut and juice was determined during the 2-minute period allowed for the juice to drain. At the end of this period, which was determined by a time clock, the wire gauze containing the sauerkraut was removed from the pan, and the weight of the juice alone was ascertained. The weight of the total solids was then determined by difference.

RESULTS

Each of the four brands of sauerkraut tested in these experiments is identified in this report by the same letter assigned to it in the study carried out previously [7] and an inferior figure 2 is added to indicate the

[7] CLOW, B., PARSONS, H. T., and STEVENSON, I. See footnote 3.

second series of experiments in the present study. The sequence of letters therefore indicates the rank of the brands in the relative concentration of vitamin C in the first study, but not, in each case, in the second study.

In carrying out the set of instructions in the four factories, some unavoidable variations occurred because of such incidents as failure of the sauerkraut to attain the required acidity in an expected time in certain of the tanks. On the whole, however, the processes of manufacture were decidedly more nearly uniform in the four factories than for the six brands tested during the preceding year, and much more detailed information was secured in the present experiment. The data for the manufacture of the four brands of sauerkraut are given in table 1.

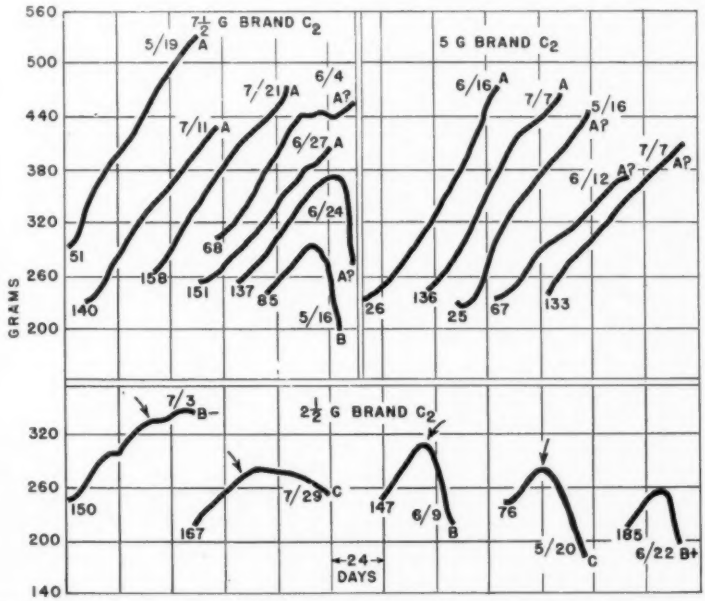

FIGURE 2.—Growth of guinea pigs and degree of protection from scurvy afforded them by 7½-, 5-, and 2½-g daily doses of commercial sauerkraut, brand C_2. For explanation of symbols see figure 1.

One hundred and thirty-one guinea pigs were used in the experiment, but only 80 are included in this report. No arbitrary selection was made of the data, however, inasmuch as all animals are included except those which were discarded for the following reasons: Failure to eat the daily portion of sauerkraut; refusal to eat the basal ration, with resultant starvation; and the presence of intercurrent abnormalities such as infected lungs or liver. In a few instances such as no. 137, figure 2, and nos. 83, 88, and 92, figure 4, the growth curves indicate some abnormality not connected with a deficiency of vitamin C, but since no specific pathological condition was detected on autopsy, the records of these animals were retained in order to make the report entirely impartial.

A problem arose in connection with attempting to secure quantitative intake of the 10- and 7½-g doses of brands F_2 and D_2, respectively, not heretofore encountered. In former experiments, the only pronounced cases of scurvy occurred on relatively small doses of sauerkraut where quantitative intake was easy to secure. In the present experiment the two brands mentioned were found to have such a low vitamin C content that it proved impossible, except in five instances, to induce quantitative intake of the 10- or 7½-g daily portions after scurvy became pronounced. At the onset of scurvy the gums and

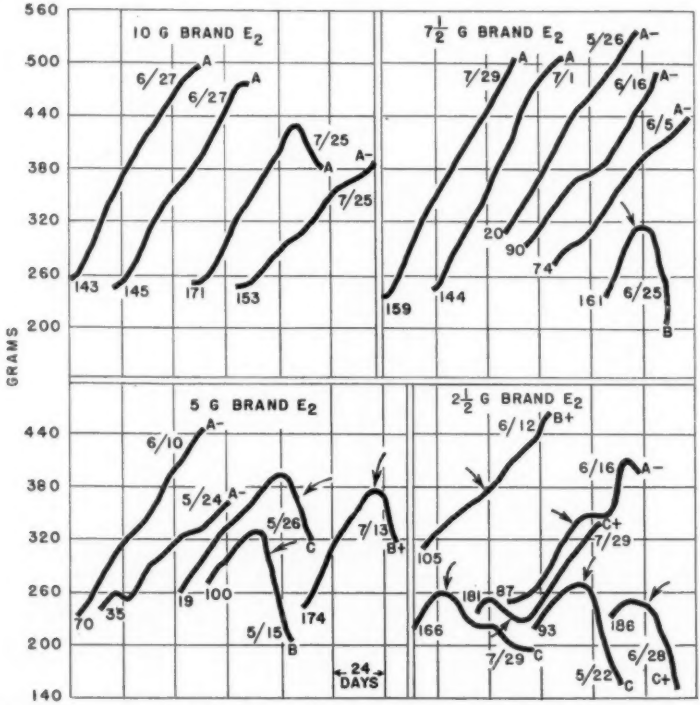

FIGURE 3.—Growth of guinea pigs and degree of protection from scurvy afforded them by 10-, 7½-, 5-, and 2½-g daily doses of commercial sauerkraut, brand E_2. For explanation of symbols see figure 1.

teeth of guinea pigs usually become tender and painful, and as a result both the total food intake and the body weight decline, as a rule. In all cases where the consumption of the daily dose of sauerkraut became irregular before the onset of scurvy, the animals were discarded. But in the case of the guinea pigs on large doses of these two brands of sauerkraut it was necessary to include the records of all animals which consumed their doses quantitatively up to the time of the appearance of severe scurvy, and to indicate the intervals of irregular intake by broken lines on the graphs. The results of the feeding experiments are presented in figures 1 to 5, inclusive.

CONTROL EXPERIMENTS

In the negative control experiment where only the basal ration was fed, both of the animals developed signs of pronounced scurvy in 16 days. The first signs of incipient scurvy occurred in 7 days.

In the positive control experiment, the six animals fed the basal ration with the addition of either 5 or 2½ g of cabbage daily were fully protected from scurvy and, except in the case of no. 49, responded with excellent growth.

The growth curves and incidence of scurvy of guinea pigs on the 7½-, 5-, and 2½-g portions of sauerkraut brand C_2 per day (fig. 2) were practically identical in this study, in which the sauerkraut was produced under controlled conditions, and that made previously under the routine procedures of the factory [8]. Protection from scurvy

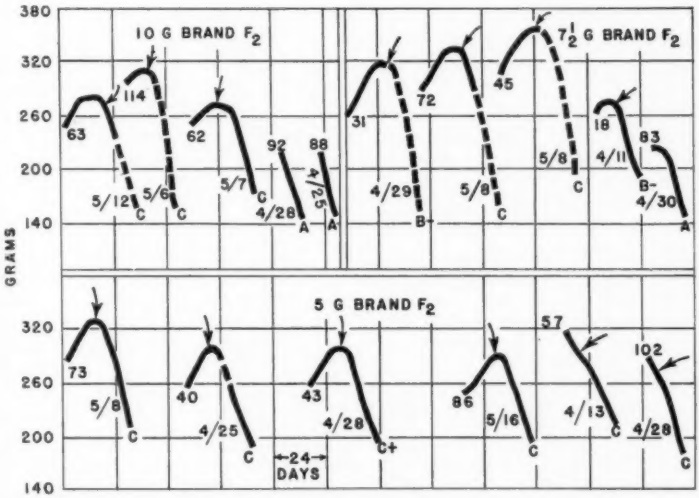

FIGURE 4.—Growth of guinea pigs and degree of protection from scurvy afforded them by 10-, 7½-, and 5-g daily doses of commercial sauerkraut, brand F_2. Broken lines indicate periods of failure to eat quantitatively the doses offered. For explanation of symbols see figure 1.

was somewhat better on the 7½-g level in last year's experiment in comparison with the present results, but was correspondingly slightly poorer on the 5- and 2½-g levels.

The protection from scurvy afforded by brand E_2 is approximately the same as for brand E from the same factory, tested previously. The results on the higher doses are slightly inferior in the present experiment but are better on the 2½-g level.

The results of the present experiments on brands F_2 (fig. 4) and D_2 (fig. 5) compare very unfavorably with those obtained in the former study on brands D and F from the same factories. In the records presented in figures 4 and 5 there is almost no indication to be found of the presence of vitamin C in the sauerkraut, even at a

[8] CLOW, B., PARSONS, H. T., and STEVENSON, I. See footnote 3.

level of 10 g daily. By a comparison with the negative control experiment in figure 1, it may be seen that scurvy occurs, on an average, as soon when these brands of sauerkraut, at even 7½- or 10-g levels, are fed daily with the basal ration, as when the basal ration is fed alone. In the case of guinea pigs 83, 88, and 92 which were fed brand F_2 and which did not develop scurvy, death ensued so quickly from some unknown cause that the symptoms of scurvy would not have become evident in so short a time had the animals been fed the basal ration only.

No data were secured for a 2½-g daily dose of brand F_2 inasmuch as the negative results of the higher levels were available before this dose was started, and it was quite evident that it would be superfluous to feed the lower amount.

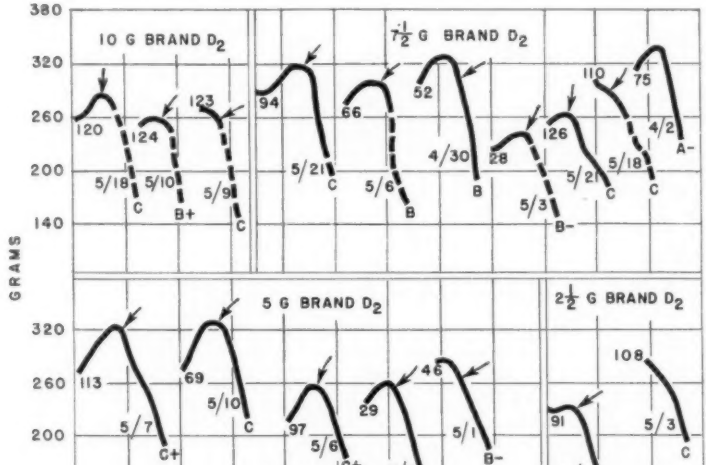

FIGURE 5.—Growth of guinea pigs and degree of protection from scurvy afforded them by 10-, 7½-, 5-, and 2½-g daily doses of commercial sauerkraut, brand D_2. Broken lines indicate periods of failure to eat quantitatively the doses offered. For explanation of other symbols see figure 1.

Although no difference can be seen in the degree of failure to protect from scurvy between brands F_2 (fig. 4) and D_2 (fig. 5), the latter is given a lower rating in the list because of the greater difficulty in inducing the guinea pigs to eat this brand, and the number of failures of animals on it for unexplained reasons. Brand D_2 was also the only one in which some of the cans showed dark streaks in the sauerkraut and even swells and leakage during the course of the experiment. These cans were, of course, rejected, and the material used for feeding was only from cans of good appearance.

DISCUSSION

Of the 4 brands of commercial sauerkraut produced under known, and somewhat uniform conditions, 2, brands C_2 and E_2, were found to contain a significant amount of vitamin C. Indeed, the richer of

these 2, brand C_2, is fairly comparable in antiscorbutic value with the specially fermented raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin.[9] Furthermore, these 2 commercial brands duplicated very closely the antiscorbutic value of the sauerkraut manufactured the previous year by the same two packers, respectively, under their routine factory conditions.[10] The other 2 brands, on the other hand, showed no demonstrable antiscorbutic potency even when fed at a 10-g level. Consumption of larger doses by the guinea pigs was not obtained, but these experiments show that if these 2 brands contain any antiscorbutic vitamin it is at least less than one fourth the amount in the 2 richer brands.

In view of the striking differences found in the vitamin C content of the 4 brands, it is of interest to compare the details of manufacturing procedure in the different plants as they are listed in table 1.

The variety of cabbage is identical only in the two richer brands. While no experiments have been reported on variations in vitamin C content of different samples of fresh raw cabbage, it is known that variety is a factor in the vitamin C content of certain fruits. Bracewell, Hoyle, and Zilva [11] have shown that one variety of English cooking apple is outstandingly superior in vitamin C content to all the other varieties tested by them. However, if All Seasons cabbage were strikingly richer in antiscorbutic vitamin than the other varieties used in these experiments, it would be expected that brand D_2, containing 70 percent of this variety, would be more nearly like brands C_2 and E_2 in antiscorbutic potency than like brand F_2. In regard to length of fermentation, temperature of fermenting vat, degree of acidity, and processes of canning, there seems to be no one factor common to both brands F_2 and D_2 as compared with brands C_2 and E_2, to account for the differences found between these two groups. It is known that vitamin C is particularly sensitive to conditions of low acidity or of alkalinity in the presence of heat. However, brand E_2, which had an outstandingly low acidity in the fermentation vat and in the can, was 1 of the 2 richer brands. Again, these 2 richer brands were the ones subjected to the greatest dilution by water or salt brine during canning, as indicated by the percentage of reduction in acidity between the fermenting vat and the sealed can. It is true that the average unfilled space in the cans of the richer brands was slightly less than in the other 2, but the degree of variation among individual cans of all 4 brands makes this appear of little significance as a gage of possible oxidation within the can after sealing.

It would seem that the differences in vitamin C content of the 4 brands must be attributable to some unidentified factor or factors which were not controlled in this experiment, but which are perhaps dependent on the regular procedure in manufacture in the different plants, inasmuch as 2 of the brands studied practically duplicated their former vitamin C content, and another brand which was previously at the bottom of the list was 1 of the 2 in which no vitamin C was demonstrated in the present experiment.

[9] Clow, B., Marlatt, A. L., Peterson, W. H., and Martin, E. A. See footnote 4.
[10] ——— Parsons, H. T., and Stevenson, I. See footnote 3.
[11] Bracewell, M. F., Hoyle, E., and Zilva, S. S. The antiscorbutic potency of apples. Biochem Jour. 24: 82-90. 1930.

SUMMARY

A study of 4 brands of commercial sauerkraut manufactured under known and fairly comparable conditions showed that 2 of these brands practically duplicated their previous antiscorbutic content, when observed under routine conditions of the factory. The other 2 brands did not show any antiscorbutic potency when fed at even a 10-g level, a record much inferior to previous results on the same brands, although 1 of these brands made an especially poor record, also, in the previous test.

No satisfactory correlation can be traced between the comparative vitamin C content in the four individual brands on the one hand, and variations in processes of manufacture on the other.

U. S. GOVERNMENT PRINTING OFFICE: 1933